Nutritional value of fresh processed and formulated diets for the green mud crab *Scylla serrata* juveniles

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OCTOBER 2006

DECLARATION

I, **UNNIKRISHNAN U.** do hereby declare that, the thesis entitled **"Nutritional value of fresh, processed and formulated diets for the green mud crab *Scylla serrata* juveniles"** is a genuine record of research work done by me under the supervision of **DR. R. PAUL RAJ**, Principal Scientist and Coordinator of Post-Graduate Programme in Mariculture, in the laboratories of the Central Marine Fisheries Research Institute, Cochin-682018, India and has not previously formed the basis for the award of any degree, diploma and associate-ship in any university.

Kochi-682018
13th October 2006

UNNIKRISHNAN U.

CERTIFICATE

This is to certify that the thesis entitled **"Nutritional value of fresh, processed and formulated diets for the green mud crab *Scylla serrata* juveniles"** is an authentic record of research work carried out by **Mr. Unnikrishnan U.** (Reg.No. 2238) under my guidance and supervision in Central Marine Fisheries Research Institute, in partial fulfilment of the requirements for the degree of Doctor of Philosophy under the faculty of Marine Sciences of the Cochin University of Science and Technology, and no part thereof has been previously formed the basis of the award of any degree, diploma and associateship in any university.

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PREFACE

Mud crabs of the genus *Scylla,* a crustacean candidate species for coastal aquaculture, is gaining importance in Asia, in the context of consecutive white spot disease outbreaks rendering shrimp culture, a risky affair. The mud crabs are fast growing, hardier species, with tolerance and adaptability towards a wide range of environmental factors and confinement, and is a high value commodity in the international sea food market. Currently mud crab culture is a small scale activity and sustained with feeding fresh and processed feed materials such as trash fish, molluscan meat and animal entrails without much scientific basis. At the present rate of growth, and once the hatchery technology is perfected, mud crab aquaculture envisages the requirement of nutritionally adequate, eco-friendly formulated off the shelf pellet feed for commercially viable farming in the near future. Among aquaculture inputs feed is a very important day- to-day requirement in mud crab farming. The present work is an attempt (1) to evaluate the efficacy of conventionally used feeds and to identify suitable feed combinations for mud crab culture, (2) to elucidate the energy budget for juvenile mud crabs, (3) to estimate the dietary protein requirements and (4) to elucidate the efficacy of various lipid supplements in formulated diets of mud crab. The thesis is organized into five chapters with a **General Introduction** in Chapter I, **Review of Literature** in Chapter II and **Materials and Methods** in Chapter III. The **Results** are presented in Chapter IV and the **Discussion** in Chapter V. The **Conclusion** of the research with salient findings and the **Summary** of the thesis follow the five chapters. The literature cited in the thesis is listed in the **Reference** section after **Conclusion and Summary** of the thesis.

CHAPTER I
Introduction

1. INTRODUCTION

The advancement in science and technology during the twentieth century has paved the way to enhance the longevity of humankind culminating in global population explosion. Food, being the basic need for sustaining health and survival, is chiefly provided by the land-based agriculture and animal husbandry sectors. Augmentation of food production from the aquatic ecosystems, which support both capture-based and culture-based production of aquatic food, is vital to meet the demand of fish protein by the growing population. The production of aquatic organisms through capture has crossed sustainable limits (Pauly and Christensen, 1995; Williams, 1997; Delgado *et al.,* 2003).

In this context, aquaculture, an age old practice of growing aquatic organisms (Bardach *et al.,* 1972; Villaluz, 1953 and Lovell *et al.,* 1978), has been established as a viable and profitable farming activity (Lovell, 1989), contributing substantially to nutritional and livelihood security. Being the fastest growing food production sector, aquaculture has registered the highest annual growth rate of 9 % which is much higher than the growth recorded in agriculture (2 %) and animal husbandry (1.2 %) sectors in 2004. The global aquaculture production has reached 51.39 million tons, contributing to 35 % of the world's total aquatic production (FAO, 2006).

Marine and brackishwater crustaceans formed only 4.56 % of the global aquaculture production and mud crabs of the genus *Scylla*, the most dominant group among the cultured portunids (60.89 %), formed a meager 4.34 % of the global farmed marine and brackishwater crustaceans. Besides, the global production of mud crabs through aquaculture has appreciated at a rate of 140 % annually, during 1994-2004, and is 5 times greater than that of the annual growth rate in shrimp culture (FAO, 2006). The growing importance of mud crab aquaculture is also evident from the fact that in 1994 the cultured crabs contributed only 30 % of the total production, whereas in 2004 contribution by aquaculture was 84 % (FAO, 2006).

Mud crabs are much sought after choice of gourmet, as a quality food item for their size, high meat yield and delicate flavour (Rattanachote and Dangwatanakul 1992) particularly in countries like Japan, Taiwan, China, Philippines and Indonesia.

In India, the export of live mud crab is a small scale trade. In the year 2000, India exported 1580 t of live mud crab and 23 t of frozen mud crab worth Rs. 260.9 million and Rs. 3.13 million respectively, as against 934 t of live mud crab (Rs. 93.5 million) and 1861 t of frozen mud crab (Rs. 229.9 million) during 1994-95 (MPEDA, 1997, 2002). The export figures clearly show that there is a shift of preference for live mud crabs as compared to frozen crabs in the international markets.

Also known as mangrove crabs, mud crabs are commonly associated with mangrove swamps and nearby intertidal and subtidal muddy habitats. As they are easily caught using very simple traps or nets and remain alive for considerable periods after capture (Gillespie and Burke, 1992) the mud crab forms an important source of income for small-scale fishers throughout the Asia-Pacific region as they are high value items.

The speciation in the genus *Scylla* has been an area of controversy and confusion, because of its wide distribution along the Indo-Pacific region, the presence of different morphotypes, and the overlapping of habitat preferences. The recent molecular taxonomy studies have confirmed at least four species under the genus *Scylla*, viz., *Scylla serrata* (green king crab), *S. tranquebarica* (pink mud crab), *S. paramamosain* (rusty mud crab) and *S. olivacea* (olive mud crab) (Keenan *et al.*, 1998) as against the earlier classification (Estampador, 1949; Radhakrishnan and Samuel, 1982; Joel and Sanjeevaraj, 1984; Kathirvel and Srinivasagam, 1992; Fuseya and Watanabe, 1996).

Mud crabs of the genus *Scylla* is also gaining importance as an alternative to tiger shrimp especially in the Asian scenario, in the context of consecutive white spot

disease out-breaks rendering shrimp culture, a risky affair (Keenan, 1999). The mud crabs, as a group, are fast growing, hardier species, with tolerance and adaptability towards a wide range of environmental conditions and confinement.

The potential for commercial aquaculture production of mud crab in the Indo-Pacific region was recognized at an International Scientific Forum held in Darwin, Australia (Keenan and Blackshaw, 1999). Mud crab culture has been conducted for at least the past 100 years in China (Yalin and Qingsheng 1994) and for the past 30 years throughout Asia. In Japan, sea ranching of hatchery-reared mud crab seeds has been practiced, but seed production has not proved reliable (Shokita *et al.*, 1991).

In India though it was a secondary or tertiary level crop in the traditional tide-fed prawn filtration and fish farming systems in the past centuries, practice of mud crab farming, where mud crab forms the primary crop is a recent development. Recurring disease out-breaks and failure of shrimp crops is prompting many farmers to diversify in to mud crab farming. Thus the mud crab farming is showing an upward trend in many coastal states of India, with a well-established fishery for commercial size as well as wild seed crabs, network of seed suppliers, farm site agents, wholesale agents and exporters to exploit the international market (Paulraj and Unnikrishnan, *In Press*).

There are mainly two types of mud crab culture systems, viz., grow-out culture and fattening culture. In mud crab grow-out culture system, juvenile crabs of $\leq$250 g size collected from the wild are stocked in brackishwater ponds and cultured for 3-8 months, to reach the desired final harvest size (Fortes, 1999; Keenan, 1999). Fattening culture is essentially the collection of newly moulted water crabs of $\geq$ 350 g, from the wild and maintaining them in brackishwater ponds, pens or in cages and feeding them till they become hard shelled. Fattening culture is a short duration venture extending up to 40 days compared to grow-out culture and has a very high return on investment (Fortes, 1999; Keenan, 1999). Mud crabs are either polycultured in brackishwater earthen ponds or monocultured in ponds,

cages, or in pens (Keenan, 1999; Trino *et al.*, 1999; Trino and Rodriguez, 2002). Trino and Rodriguez (2002) have also discussed the profitability of monosex culture of the *Scylla* sp. and found it promising.

The major constraint restricting further expansion of mud crab culture is the limited supply of crab seed for stocking enclosures (Keenan, 1999). Globally almost all mud crab aquaculture production relies on wild-caught stock, as larval rearing has not yet reached a commercially viable level for stocking into aquaculture farms (Cowan, 1984; Liong, 1992), and in India the farming is exclusively dependent on the wild caught seeds (Marichamy and Rajapackiam, 1998; 2001). Kerala, being a major hub of mud crab aquaculture in India, with rich mangrove systems, faces severe shortage in seeds from the wild. Andhra Pradesh and Tamil Nadu, the east coast states of India, are the major sources of wild caught seeds for the mud crab farming in Kerala during peak farming seasons, from September to January, with a well organized seed collection, transportation, and marketing network (Paulraj and Unnikrishnan, *In Press*).

Like any other aquaculture production system feed forms a very important input in mud crab farming. But in practice the relevance of feed is yet to be realized by the crab farmers (Paulraj and Unnikrishnan, *In Press).* At present, the concern is only for the cheapest feedstuff and investment on feed is around 5-6 % of the total expenditure in the case of fattening. This clearly indicates that the quality of feed is compromised for price and also for the ease of procurement.

Lijauco *et al.* (1980) observed that mud crabs could not be reared on a diet composed solely of fish since this diet resulted in slow growth rate and poor condition. Various studies on food preference and feeding habits showed that mud crabs require both molluscan and crustacean material in their diets (Hill, 1976; Joel and Sanjeevaraj, 1986; Prasad and Neelakantan, 1988; Jayamane and Jinadasa, 1991; Lee, 1971; Yalin and Qingsheng, 1994, Marasigan, 1999). Heasman and Fielder (1983) have highlighted the need for studies on feed form, texture, size,

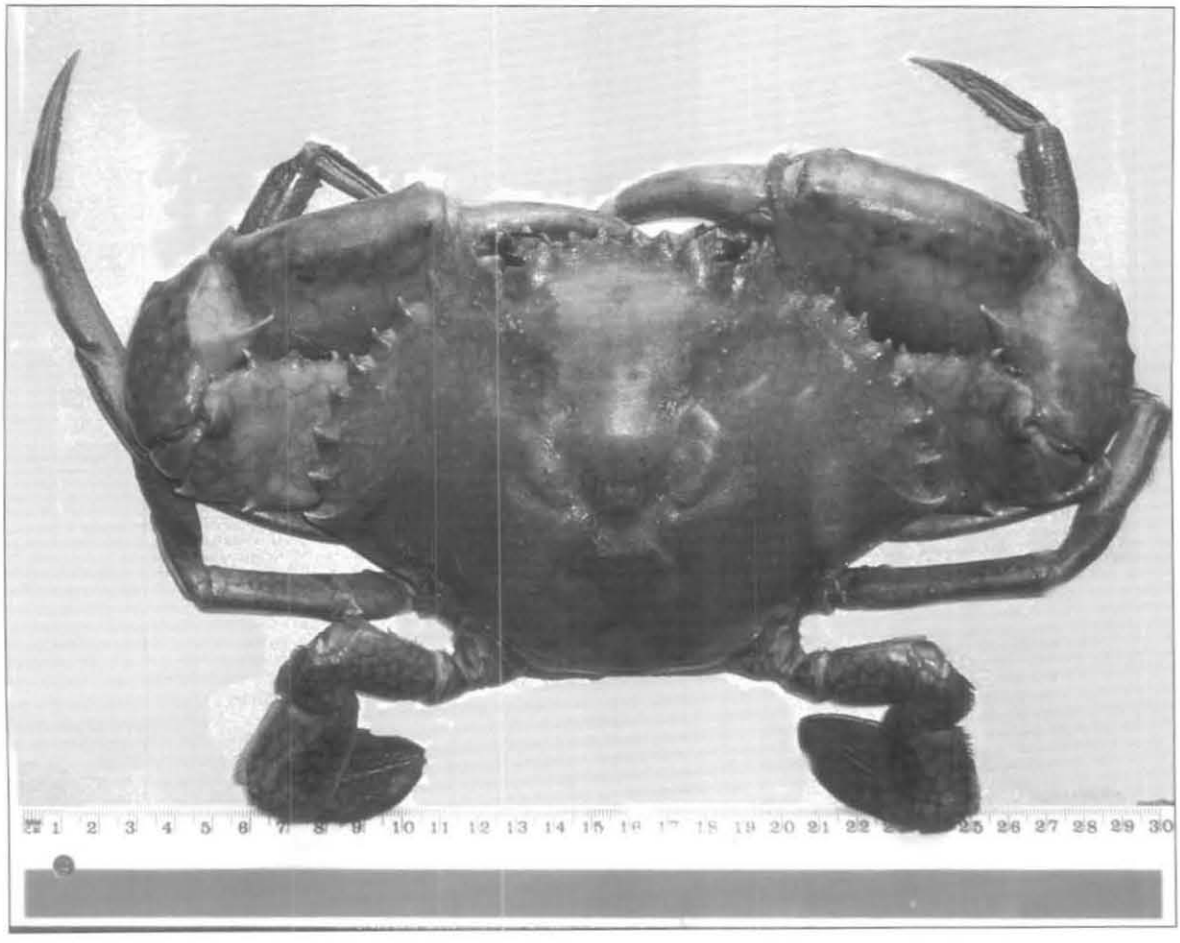
odour, method of feed distribution, and on feeding behaviour of mud crabs at different life stages.

The fresh and natural feeds like trash fish and molluscan meat cannot support the expansion of commercial mud crab culture because of limited availability, nutritional inadequacy and likely impact on water quality deterioration (Paulraj and Unnikrishnan, *In Press*). For sustainable and commercial scale aquaculture production, formulated feed is an essential component. The development of a successful cost-effective formulated feed, which replaces the natural feed and satisfy the nutrient requirement of the organism under captivity, requires comprehensive information on the feeding habit, feed preference, nutrient and energy requirement etc. of a particular species. Studies on nutritional requirements of mud crabs in captivity are critical to the development of the mud crab aquaculture industry (Marasigan, 1999). To date, there is very limited information available on the nutrient requirements of mud crab (Sheen and Wu, 1999; Sheen, 2000; Catacutan, 2002; Catacutan, 2003; Sheen and Wu, 2003). Besides defining the individual nutrient requirements, it is equally important to formulate combinations of commonly available ingredients for optimum performance with respect to growth and survival (Hutabarat, 1999).

Taking consideration of the facts discussed above, five priority areas were identified and fixed as objectives of the present work as listed below.

- To assess the feed and feeding practices in mud crab farming in Kerala
- To evaluate the efficacy of selected fresh, frozen and processed diets
- To elucidate the energy budget
- To determine the dietary protein requirement of juvenile mud crabs using formulated diets.
- To determine the efficacy of selected lipid supplements in formulated diets.

PLATE-1



Green mud crab *Scylla serrata* (Keenan *et al.*, 1998)

CHAPTER II
Review of Literature

2. REVIEW OF LITERATURE

2.1 Feeding behaviour

Traditionally mud crab has been viewed as a carnivore showing preference for natural diets containing molluscs, crustaceans, fish and dead animal matter with stray occurrence of plant-based materials (Hill,1976 and 1979; Prasad and Neelakantan, 1988; Joel and Sanjeevaraj, 1986; Prasad *et al.*, 1988). According to Jayamane and Jindasa (1991) juvenile mud crabs are omnivores that feed primarily on crustaceans and the sub-adults are carnivores that prey on bivalves. The occurrence of various prey species in the gut content of mud crab reflects the relative abundance of that particular prey species in the habitat (Ensis, 1973) and also suggests their adaptation to diverse niches and wide-spread distribution in the Indo-Pacific region (Hill, 1979).

Studies on the morphology of oral appendages, histology of alimentary tract, digestive enzyme profile and digestive physiology, as well as the feeding experiments with formulated diet show that the mud crab can make use of a wide variety of feed materials of plant and animal origin with better digestibilities (Catacutan *et al.,* 2003; Pavasovic *et al.*, 2004).

Contradictory statements have been made on the ability of mud crab to predate on faster moving preys, particularly fishes (Prasad and Neelakantan, 1988) and their limited ability to do so with a preference on sessile or slow moving benthic invertebrates, particularly molluscs (Hill, 1976). The latter is in consensus with the findings of Muntz *et al.* (1965) on the feeding behaviour of 3 species of crabs in British waters, crabs which exhibited more preference towards slow moving or sessile preys. However observations of Joel and Sanjeevaraj (1986) and Warner (1977) revealed that the juvenile mud crabs capture fast moving prey like small fishes and shrimp, and have attributed this to their slim sharp toothed chelae with a relatively high proportion of fast contracting muscles adapted for the rapid movements, and also to the powerful thrust movements of swimmerets (Hill,1976).

Joel and Sanjeevaraj (1986) have attributed the nocturnal locomotor activity in the wild adult mud crab to its nocturnal feeding habit.

2.2. Feeds and Feeding in mud crab culture

Review of literature on the feed management in mud crab farms clearly indicate that the feeds used in crab farming are generally locally available cheaper items such as trash fish, animal entrails and rarely molluscan meat. The feeds and feed management in mud crab farms in various countries including India, till date is summarized and presented in Table-1.

2.3. Experimental feeding trials in *Scylla* spp.

Preliminary feeding trials have been made for evaluating the suitability of locally available fresh and processed feeds in laboratory scale as well as at farm level and assessment of growth response and reproductive performance of *Scylla* spp. and efforts have been made to formulate suitable compounded diets, and the results have been highlighted in Table-2.

2.4. Nutritional requirements

2.4.1. Energy requirements

The total energy requirement of an animal is the sum of maintenance energy requirement for basal metabolism, energy requirement for growth, and the energy expenditure on reproduction (Proser and Brown, 1965). The ratio between energy channeled into growth and metabolism represents the efficiency of energy utilization (Duncan and Klekowsky, 1975) and may be a useful index for studies of energy transfer in the ecosystem for the optimization of culture techniques. Information on energy utilization is essential to develop cost-effective diets because energy must be supplied in sufficient amounts so that protein is almost exclusively spared for tissue synthesis. However, information about the utilization of energy-yielding nutrients in crustaceans is still sparse and the problem in providing dietary energy to crustaceans primarily resides in their inability to tolerate greater than about 10% of lipid in their diets, leaving carbohydrates as the non-protein energy source to be used in diet formulations (Cuzon and Guillaume, 1997). There is considerable

Table-1: Feeding trials in *Scylla* spp.

Investigator	Country	Objective	Result
Raman *et al.* (1980)	India	Performance evaluation of feeds of plant (sea grass *Halophila ovalis* and filamentous algae *Chaetomorpha* sp. and *Enteromorpha* sp.) and animal origin (trash fish and gastropods) for *Scylla serrata*.	Mixed ration of plant matter and animal matter at 1:1 gave a growth performance comparable with animal matter alone; with plant matter alone poor produced performance
Cheong *e tal.* (1992)	Srilanka	Evaluation of modified shrimp feed formulations vs. frozen clam (*Meretrix casta*)	Growth response of crabs fed the shrimp feed was inferior to that of clam fed.
Fortes (1999)	Thailand	Pond grow-out experiments of *S. serrata* in mangroves, with trash fish fed at 3 % of biomass daily	30-60 g juvenile crabs gained 86-160 g in 125 days
Hutabarat (1999)	Central Java	Survey on the suitability of locally available raw materials for the mud crab feed formulation	Listed the nutritive value of a number of suitable raw materials and also formulated the diets containing 30-35 % crude protein. Details on the feeding trial is not available.
Genodepa (1999)	Philippines	Comparison of growth and survival of *S. serrata* pen culture experiments in mangroves fed trash fish fed daily at 3% of biomass split into two feedings at twilight hours and without feeding	Growth rates were found to increase in unfed group, with drastic decline of survival due to severe cannibalism.

Millamena and Quinitio (2000)	Philippines	Reproductive performance and larval quality in wild caught *Scylla serrata* fed with artificial and natural diets alone and in combination	The artificial diet in combination with fresh feed gave the best larval survival and is attributed to the vitamins and essential fatty acids in the artificial diet.
Millamena and Bangcaya (2001)	Philippines	Reproductive performance and larval quality in pond raised *Scylla serrata* fed with artificial and natural diets alone and in combination	The results were similar to the previous study on wild caught *Scylla serrata*
Catacutan *et al.* (2003)	Philippines	Digestibilty of locally available feed ingredients suitable for feed formulation for *S. serrata* juveniles	Advocates the suitability of more plant stuffs in feed for *S. serrata* for its ability to utilize carbohydrate rich feed stuffs compared to protein rich feed stuffs. Also listed a wide range of plant and animal feed stuffs suitable for mud crab feed feed formulation based on digestibility.
Paulraj and Unnikrishnan (2003)	India	Formulated pellet feed for fattening culture of *S. serrata*	Feeding with formulated pellet feed could considerably reduce the fattening period as compared with salted trash fish.
Rodriguez *et al.* (2003)	Philippines	Effect of diet and harvesting regimen on production of *S.olivacea* in grow-outs.	Mixed feeding with gastropods and cooked corn resulted in better performance than the fish by-catch

Unnikrishnan and Paulraj (2003)	India	Efficacy of fresh and processed feeds for juvenile *S. serrata*	A mixed ration of bivalve meat, shrimp head and fish gave the best growth response than the individual diets.
Christensen *et al.* (2004)	Vietnam	Performance of mud crabs fed between trash fish, sesarmid crabs and natural productivity based grow-outs in mangrove ponds.	Though the response of crabs in treatments were not significantly different, the fed crabs exhibited better growth.
Unnikrishnan and Paulraj (2005)	India	Efficacy of lipid supplements viz., cod liver oil, sunflower oil and soybean oil alone and in combinations in formulated diet of juvenile crabs	The growth response obtained with cod liver oil and plant oils in 1:1 ratio was not significantly different from that obtained with cod liver oil alone, but plant oils supplement alone in feeds resulted in poor response than the other feeds
Dunaidah *et al.* (2003)	Indonesia	Reproductive performance and offspring quality in mud crab *(Scylla paramamosain)* fed fresh feeds (squid, shrimp, trash fish, *Artemia* biomass etc.) and shrimp feed	Formulated diets resulted in better larval survival than fresh feeds, for its superior nutritional composition matching the requirement of crabs. Further, manipulation of the nutritional profile in formulated diet is easier than the other diets.

Table- 2: Feeds and feeding in mud crab culture

Reported by	Country	Feeding strategy	Feeds used
De Silva (1992)	Srilanka	Daily, not specified	Offal and bones with meat, clam, trash fish.
Ladra (1992)	Philippines	Daily, 1-2 times, 5-8 % of biomass	Kitchen leftovers like rice, vegetables, fish and animal entrails, chopped trash fish, African snails, fruit peelings, mussel
Liong (1992)	Malaysia	Not specified	Fresh, salted or sun dried trash fish and tuna fish offal
Prinpanapong and Youngwanichsaed 1992)	Thailand	40 % of biomass once daily	Horse mussel
Rattanachote and Dangwatanakul (1992)	Thailand	Daily 2 times, 7-10% of biomass.	Trash fish and horse mussel
Samarasinghe *et al.* (1992)	Srilanka	Daily, 10 % and 5 % of biomass to juvenile and adults respectively	Chopped trash fish or shrimp heads.
Srinivasagam and Kathirvel (1992)	India	Not specified	Clam meat, mussel meat and trash fish
Yalin and Qengsheng (1994)	China	Not specified	Freshwater molluscs and fish bycatch
Dat (1999)	Vietnam	Grow-out: 4-6 % of biomass, once daily in the evening hours. Fattening: 5-8 %, once daily Soft-shell crab production: 2-4 % of biomass, split rationing at twilight hours.	Raw and fresh, crushed fish, small crabs, oysters, molluscs, shrimp or fish heads etc.
Marichamy and Rajapakiam (2001)	India	10-15 % of the biomass daily for the production of mature females	Fresh bivalve meat

variation in the utilization of carbohydrate sources by crustacean species, making it difficult to calculate accurately the true energy value of diets. In addition, high levels of glucose, a primary monomer of carbohydrates, are not tolerated well by crustaceans (Cuzon and Guillaume, 1997).

2.4.1.1. Energy partitioning

In crustaceans, ingested energy *(C)* is primarily channeled for growth *(P)*, metabolism and maintenance *(R)*, excretion *(U)*, faeces *(F)* and exuvia *(E)*, following the expression $C = P + R + U + F + E$ (Petrusewicz and Macfadyen, 1970; Mootz and Epifanio, 1974; Levine and Sulkin, 1979).

The ratio between energy channeled into growth and metabolism represents the efficiency of energy utilization (Duncan and Klekowsky, 1975) and may be a useful index for studies of energy transfer in the ecosystem, and for the optimization of culture techniques. In decapods, this ratio may vary in function of the ontogenetic development (Mootz and Epifanio, 1974; Logan and Epifanio, 1978; Levine and Sulkin, 1979; Pederson and Capuzzo, 1984), environmental conditions (Dawirs, 1983) and nutritional state (Dawirs, 1983, 1987).

The energy expended in metabolic processes, measured by oxygen consumption *(R)*, is used for the maintenance of physiological functions including locomotion, feeding, food processing, and for the synthesis of new tissue (KiØrboe and MØhlenberg, 1987). Growth *(P)* may be considered as the energy materially gained by the individual and can be stored as body reserves (Kurmaly et al., 1989). Energy loss by excretion *(U)*, which is estimated in terms of ammonia excretion and exuvia *(E)*, was less representative compared to the energy expended in growth and metabolism (Logan and Epifanio, 1978; Johns, 1982; Pederson and Capuzzo, 1984).

2.4.1.2. Dietary energy and growth

The dietary energy level in formulated diets for various crustaceans in general lies between 13 and 17 MJ/kg of diet (Cuzon and Guillaum, 1997). The

early postlarvae of *L.* vannamei and *L. setiferus,* fed with individual or a mixed diet of *Artemia*, microparticulate commercial diet, *Tetraselmis* and *Chaetoceros*, received 0.567 to 2.2318 J/day as ingested energy, of which the greater portion was utilized for growth in the mixed diet treatment in contrast to the individual feed treatments (Brito *et al.*, 2004). Energy partitioning studies in *Farfentopenaeus paulensis* show that energy diverted for growth increased from protozoea I to III reaching the maximum, and further decreased through mysis I to III (Lemos and Phan, 2001). In *Panulirus homarus*, Anilkumar (2002) reported an intake energy rate of 235.67 J/g body weight/day at 24-27 °C with an assimilation efficiency of 97.93%, of which allocation for growth ranged between 8 and 10 % of the total intake energy, when fed clam meat. *Carcinus maenas* fed *Mytilus edulis* meat exhibited an intake energy rate of 15.65 kJ/day for crab of 25.1 g body weight at 25 °C, of which 56.69% was reported as scope for growth (Osha *et al.*, 2002).

2.4.1.3. Moulting

Most of the energy requirement trials were carried out in larval, postlarval and juvenile shrimps and prawns wherein exuvial recovery is difficult since in most cases the animals will consume the exuvia after moult to recoup the mineral and energy loss (Kurmaly *et al.*, 1989). But in larger shrimps, and juveniles and adults of lobsters and crabs, the possibility of recovery is more wherein the feeding activity is very much reduced immediately after moulting. The exuvial loss in crustaceans is assumed to be 5% of the total energy content of the body (Kurmaly *et al.*, 1989; Carvalho, 1992; Lemos and Phan, 2001). In Indian rock lobster, *Panulirus homarus* the exuviae formed about 3 % of the total intake energy (Vijayakumaran and Radhakrishnan, 1984; Anilkumar, 2002).

Bio-energetic evaluation of the crab *Cancer pagurus* fed on *Mytilus edulis,* revealed that 27.42% of ingested energy is spent towards excretion (E) and defaecation (D), 15.89% towards metabolism (R) and about 56.69% is deposited as growth increment (Osha *et al.*, 2002). Lemos and Phan (2001) elucidated the energy budget of *F. paulensis* larvae and reported an expenditure of 42% of intake

energy towards growth, 52% towards metabolism and the remaining towards excretion and defaecation.

The relationship between salinity, temperature and excretion of ammonia nitrogen in *Penaeus japonicus* (Chen *et al.*, 1994) and *Xiphopenaeus kroyeri* (Carvalho and Phan, 1997) shows that any deviation from the optimum range of salinity causes an increase in ammonia excretion, where as in the case of temperature fluctuation there exists a linear relation.

2.4.1.4. Food composition and energy balance

Recent research has demonstrated that shrimps fed a diet with 40% protein were energetically more efficient than those fed diets with 15 or 5% protein levels. A lower respiratory metabolism and, in consequence, a higher energy retention was observed in shrimp fed high dietary protein levels than with low levels (Pascual *et al.*, 2004). The nutritional trials with *Penaeus vannamei* indicate that dietary protein is the regulatory nutrient in crustaceans particularly in the energy balance (Rosas *et al.*, 2002).

2.4.1.5. Specific Dynamic Action and Basal Metabolic rate

The increase in oxygen uptake that follows feeding, termed specific dynamic action (SDA), has been described in a variety of animals from vertebrates, such as fish, reptiles, and mammals to invertebrates, such as crustaceans and molluscs. In crustaceans, SDA has been described for a number of species including brachyuran crabs (Aldrich, 1975, Houlihan *et al.*, 1990, McGaw and Reiber, 2000; Robertson *et al.*, 2001b); natantian shrimps (Hiller-Adams and Childress, 1983; Nelson *et al.*, 1985); isopods (Newell *et al.*, 1976; Carefoot, 1987, 1990); and amphipods (Chapelle *et al.*, 1994). In each case the SDA response is characterized by a steady rise in rates of oxygen uptake after a meal, over and above the rates measured in resting fasted animals, and a gradual decline back to pre-feeding values over a period of several hours to days. Three parameters of the SDA response are usually described including the overall magnitude, the integrated post-prandial increase in

oxygen uptake, the peak levels and the duration (Jobling and Davies, 1980). Although peak SDA values are typically two to four times higher than pre-feeding standard rates of oxygen uptake in marine ectotherms, the time taken to reach a maximal SDA level, the absolute increment and the duration of the SDA response can vary considerably (Peck, 1998).

The factors known to influence SDA variables in crustaceans include endogenous factors, such as changes in meal size and composition, body size, levels of activity and environmental factors, such as salinity and temperature (Newell *et al.*, 1976; Houlihan *et al.*, 1990b; Burggren *et al.*, 1993; Peck, 1998). SDA reflects the energy requirements of numerous physical and biochemical processes involved with digestion (Brody, 1945; Beamish, 1974). These include movement and handling of food, absorption and storage of nutrients, deamination of amino acids, synthesis of excretory products and the increased synthesis of proteins and lipids associated with growth (Jobling, 1992; Wieser, 1994). A number of physiological responses, such as cardiovascular adjustments, also accompany a feeding event, as seen in the blue crab, *Callinectes sapidus* (McGaw and Reiber, 2000). Cardiac output, for instance, increases immediately upon the detection of food due to an increase in stroke volume, and remains elevated for 16-18 hrs, while the food passes through the digestive system. In addition, haemolymph flow increases in the arteries supplying the mouth-parts, gut muscles and hepatopancreas. While it is difficult to assess the metabolic cost of these physiological changes, estimates on the mechanical cost of digestion are calculated to be 5-8% of SDA (Carefoot, 1987, 1990). In addition, there is growing evidence to suggest that protein synthesis is an important contributor to SDA in crustaceans. This is shown by the proportional increase in whole animal fractional rates of protein synthesis and oxygen uptake rate following a meal in isopod and decapod crustaceans (Houlihan *et al.*, 1990b, Robertson *et al.*, 2001a,b,c). In fact, it was estimated that protein synthesis accounts for 20-37% of the rise in oxygen consumption measured in the shore crab, *Carcinus maenas*, after a meal (Houlihan *et al.*, 1990b). Interestingly the reduction in tissue accretion of 65% following one week of starvation in *Carcinus maenas* (Houlihan *et*

al., 1990b) correlates with the previously reported 60% reduction in oxygen uptake rates (Wallace, 1973), providing further evidence for a close relationship between rates of protein synthesis and metabolism. As temperature can influence both metabolic rate and rates of protein synthesis in fasted and inactive crustaceans (Whiteley *et al.*, 1996; El Haj and Whiteley, 1997) it is likely to have an important effect on SDA, and therefore protein utilization and growth. Previous studies on fish with similar lifestyles, but from different thermal regimes polar, temperate, and tropical, indicate that SDA variables change with habitat temperature (Johnston and Battram, 1993). Polar fish exhibit a slower SDA response than temperate and tropical species, due to a smaller absolute incremental post-prandial rise in oxygen consumption and an extended SDA duration. The slower SDA response at low temperatures was attributed to low rates of oxygen uptake and low metabolic capabilities after feeding (Boyce and Clarke, 1947).

2.4.2. Carbohydrates

Carbohydrates are polyhydroxy aldehydes or ketones, or substances that yield such compounds on hydrolysis, and the most abundant biomolecules on earth central to the energy yielding non-photosynthetic pathways and key energy trapping molecules in primary production (Lehninger *et al.*, 1991). Carbohydrates exist in simple forms such as mono, oligo or polysaccharides, and complex forms like, glycoproteins and glycolipids with varied functions (joint lubricant, cell adhesion, transport, osmoregulation etc.) in organisms. Water insoluble carbohydrate polymers have important structural and protective function (bacterial and plant cell wall, chitin in the exoskeleton of crustaceans) in biological system (Lehninger *et al.*, 1991).

The utilization of carbohydrate sources by aquatic animals varies and seems to be less efficient than terrestrial domesticated animals and the information about the carbohydrate nutrition in crustaceans is very much limited (Shiau, 1997). When compared to fin fishes, simple sugars are poorly utilized by crustaceans. The majority of the available information is centered on shrimp species exclusively, and

research is focused on the protein sparing action of carbohydrates (Sick and Andrews, 1973; Catacutan, 1991).

From the table-3, it is evident that the shrimps are more able to utilize dimeric or polymeric carbohydrates than the monosaccharides and the best utilization and tolerance levels for carbohydrates lies between 20-30 % in the diets (Alava and Pascaul, 1987; Catacutan, 1991).

The mechanism responsible for the poor utilization of glucose by species of crustaceans is not fully understood. Being a simple sugar, glucose requires no further enzymatic degradation and therefore rapidly gets absorbed across the digestive tract resulting in sudden surge in haemolymph as a result of insufficiency of glucose metabolizing enzymes further causing "negative physiological effects" and results in poor utilisation of glucose in the diets of rainbow trout and carp (Piefer and Pfeffer 1980; Furuichi and Yone 1982a, 1982b; Murai *et al.* 1983). A similar situation may exist in crustaceans (Shiau, 1997).

Apart from the carbohydrates discussed above chitin is an important structural component of crustacean exoskeleton and a 0.5% level recommended in shrimp feeds is reported to have growth promoting effect (Akiyama *et al.*, 1992). Kitabayashi *et al.* (1971) found that the addition of 0.52% glucosamine, monomer of chitin, in diets improved the growth, but the addition of chitin retarded the growth. Contradicting this Deshimaru and Kuroki (1974b) have stated that supplementation of glucosamine in diet of *M. japonicus* is not required and the presence of glucosamine interferes with the growth promoting effect of cholesterol.

Table-3: Carbohydrate utilization by penaeid shrimp

Species	Carbohydrate source	% Tested	Results	Reference
Penaeus setiferus	Glucose, starch	0, 20, 30, 40	Starch- best utilized, with good growth; glucose- poorly utilized with poor growth	Andrews *et al.* (1972)
Penaeus duorarum	Glucose, starch	10, 40	40 % starch produced better response than 40 % of glucose	Sick and Andrews (1973)
Penaeus japonicus	Glycogen, starch, dextrin, glucose, sucrose	10	Highest weight gain for sucrose; highest feed efficiency for starch; poor utilization of glucose	Deshimaru and Yone (1978b)
Penaeus japonicus	Glucose, starch, dextrin, potato starch, glycogen. galactose, fructose, sucrose, maltose	19.5	Poor utilization of monosaccharides (glucose, galactose)	Abdel-Rahman *et al.* (1979)
Penaeus monodon	trehalose. sucrose, glucose	10, 20, 30	Higher weight gain and survival with trehalose and sucrose	Alava and Pascual (1987)
Penaeus monodon	Wheat flour first grade, second grade	35	No difference in utilisation	Shiau *et al.* (1991)
Penaeus monodon	Gelatinized bread flour	5 - 35	The best utilization and tolerance found at 25%, considerable reduction in weight gain and FCR at 35%	Catacutan (1991)
Penaeus monodon	Glucose, dextrin, starch	20 - 30	Best SGR, FCR and PER with corn starch and dextrin than glucose, starch protein sparing than glucose	Shiau and Peng (1992)
Penaeus indicus	Glucose, fructose and galactose, maltose sucrose, glycogen and starch	22.5	Best growth performance	Ali, 1982

2.4.3. Dietary protein and amino acids

Proteins are the major organic material in animal tissues, constituting about 65 to 75 % of the body mass on dry matter basis and have varied functions, from structural to catalysis. The scarcity of carbohydrates and the abundance of lipids and proteins are attributed to the common adaptive trend of aquatic organisms to use protein as energy source. The protein requirement of aquatic animals is higher than that of terrestrial animals, as they are ectothermic and ammonotelic (Guillaume, 1997).

2.4.3.1. Dietary protein level

The term, protein requirement has been defined as the minimum or the maximum tolerance needed per animal per day, where as the maintenance requirement for protein can be defined as the level of protein required for maintaining body functions associated with protein metabolism, with all other nutrients having been provided in adequate amounts (Guillaume, 1997).

The dietary protein requirement of farmed crustaceans is an important nutritional consideration and received much attention because protein is a major limiting nutrient for growth and is one of the primary cost components of commercially prepared feeds (New, 1976; Smith *et al.*, 1985; Akiyama *et al.*, 1992; Kureshy and Davis, 2002). Any reduction in dietary protein level that does not negatively affect crustacean growth would substantially reduce the feed cost. Most of the available data related to growth, dietary protein level and protein utilization of crustaceans have been reviewed by New (1976), Cuzon *et al.* (1994), D'Abramo and Sheen (1994) and Conklin (1995). The optimum protein level for important farmed crustaceans, such as shrimp, prawn and lobster ranges from 28 to 60% (Akiyama *et al.*, 1992; Conklin, 1995).

Protein requirement has been reported to be affected by changes in biotic factors (e.g., species, physiological state, size) and dietary characteristics (e.g., protein quality and energy:protein ratio). Abiotic factors such as temperature and salinity may also affect the protein requirement (Guillaume, 1997).

The protein requirement of a given species is often determined based on the response (e.g., weight gain, feed efficiency, protein conversion efficiency) of the animal to varying levels of dietary protein under a given set of circumstances and therefore, the protein requirement is better described as the optimal protein content required in the diet to produce best response by the animal (Kureshy and Davis, 2002). Additionally, protein content of the feed and dietary availability can affect water quality via nitrogen excretion. Protein that is utilized for energy and not deposited for growth contributes to the release of nitrogen metabolites into the culture medium (Cho *et al.*, 1994).

Attempts have been directed towards evolving criteria other than optimal growth for the evaluation of protein requirement. Chuang *et al.* (1985) have found that the dietary protein requirement increases with proteolytic activity, suggesting that the gross protein requirement is positively correlated with the capacity for protein digestion. This relationship, however, is yet to be confirmed.

Investigations on the molt frequency and duration of inter-molt period in crustaceans in relation to the dietary protein level revealed that except for *Crangon crangon* (Regnault and Luquet, 1974) no significant relationship could be established, particularly with regard to moult frequency (Regnault and Luquet 1974; Huner and Meyers 1979; Lucien-Brun *et al.*, 1985). Apparently, protein level, like other nutritional factors, principally influences growth via weight gain at molt, rather than molt frequency.

Very few studies have been conducted to evaluate the influence of dietary protein on protein content of body tissue. The highest dietary body protein content was obtained at "over optimal" crude dietary protein levels in *Homarus gammarus* (Lucien-Brun *et al.,* 1985) and *Penaeus monodon* (Alava and Lim, 1983). Biochemical criteria (DNA content, wet biomass/DNA) were proposed, but very seldom used (Lucien-Brun *et al.*, 1985; Thomas and Diwan, 1993). Weight gain or specific growth rate remains by far the most common reliable performance indicator.

The optimal protein levels, measured by growth responses of different species of crustaceans are presented in Table-4. These levels vary from 50-55% for *Penaeus japonicus* and *P penicillatus* to 25-30% for *P. aztecus* and *Macrobrachium rosenbergii.* Overall, these protein levels are high, similar to the range observed in species of finfish (Tacon and Cowey, 1985). Several authors have interpreted the apparently wide inter-species differences in protein requirement to be the result of the evolution and adaptation to specific feeding habits. The protein requirement would be lowest in herbivorous species such as *Penaeus vannamei* and highest in typically carnivorous species such as *P. japonicus* (Kanazawa, 1990) and that the high proteolytic activity in the digestive tract is correlated with a high protein requirement in carnivorous species (Chuang, 1990). Also, differences in optimal requirements partly reflect the influence of other factors such as life stage and size (Kanazawa, 1990; Shiau *et al.*, 1991). Smaller animals require higher dietary levels as documented for *Crangon crangon* (Regnault and Luquet, 1974), *Penaeus brasiliensis* (Liao *et al.*, 1986) *P. californiensis, P. stylirostris* and *P. vannamei* (Colvin and Brand, 1977) and may be attributed to the fact that, the specific growth rate decreases as size increases. Accordingly, Akiyama *et al.* (1992) recommended a reduction in the dietary protein level from 45% (for postlarvae below 0.5 g) to 36% for shrimp exceeding 15 g. Dietary protein level and specific growth rate are highly correlated in fish (Tacon and Cowey, 1985), but this relationship has yet to be studied in crustaceans.

Table-4: Dietary protein requirement of shrimps, lobsters and crabs

Species	Source of protein	Protein (%)	Author(s)
Penaeus monodon	Casein + fish meal	46	Lee (1971)
Penaeus monodon	Casein	40	AQUACOP (1978)
Penaeus monodon	Mixture	35	Shiau *et al.* (1991)
Penaeus japonicus	Squid meal	60	Deshimaru and Shigueno (1972)
Penaeus japonicus	Shrimp meal	> 40	Balazs *et al.* (1973)
Penaeus japonicus	Casein + albumin	54	Deshimaru and Kuroki (1974a)
Penaeus japonicus	Casein + albumin	52	Deshimaru and Yone (1978c)
Penaeus japonicus	Crab protein	42	Koshio *et al.* (1993a)
Penaeus indicus	Shrimp meal + yeast	43	Colvin, (1976a)
Penaeus indicus	Lipid free casein, gelatin andd egg albumin fortified with amino acids	35 - 40	Gopal and Paulraj (1990)
Penaeus merguiensis	Mixture of animal and plant ingredients	50	AQUACOP (1978)
Penaeus aztecus	Fish meal + squid meal	29-31	Shewbart and Miles (1973)
Penaeus duorarum	Soybean meal	30	Sick and Andrews (1973)
Penaeus aztecus	Fish meal + mixture	<40	Venkataramiah *et al.* (1975)
Penaeus kerathurus	Mxture of animal and plant ingredients	>40	Fernandez and Puchal (1979)
Penaeus californiensis	Mixture of animal and plant ingredients	> 44	Colvin and Brand (1977)
Penaeus brasiliensis	Shrimp meal + casein	45-55	Liao *et al.* (1986)
Penaeus vannamei	MIture of animal and plant ingredients	30	Cousin *et al.* (1993)
Metapenaeus monoceros	Casein	55	Kanazawa *et al.* (1981)
Metapenaeus macleayi	Casein	27	Macguire and Hume (1982)

Metapenaeus japonicus	Casein + albumin	45-55	Teshima and Kanazawa (1984)
Crangon crangon	Fish protein hydrolysate	30-60	Regnault and Luquet (1974)
Homarus americanus	Mixture of animal and plant ingredients	20-23	Capuzzo and Lancaster (1979)
Homarus gammarus	Fish meal + shrimp meal + wheat gluten	35	Lucien-Brun *et al.* (1985)
Procambarus clarkii	Mixture of animal and plant ingredients	20-30	Huner and Meyers (1979)
Eriocheir sinensis	Mixture of animal and plant ingredients	42	Mu *et al.* 1998

These values may be influenced to some extent by species, size, protein quality and the level of non-protein energy in the diet and also by environmental conditions. The re-evaluation of protein requirement of *Penaeus japonicus* (Koshio *et al.*, 1993a) show that, with crab protein based diets the requirement does not exceed 42%, a level lower than the range of 52-57% previously determined with casein and albumin as protein sources (Deshimaru and Shigueno, 1972; Deshimaru and Yone, 1978c). The investigations on nitrogen balance in growing crustaceans indicate that the best net protein utilization (NPU) and productive protein values (PPV) were observed for the slightly lower dietary protein level than the absolute requirement (Millikin *et al.*, 1980; Shiau *et al.*, 1991).

2.4.3.2. Dietary amino acid requirements

The incapability of crustaceans, particularly shrimps to utilize dietary crystalline amino acids had made it a barrier in quantifying their essential amino acid requirements (Guillaume, 1997). Earlier studies on amino acid requirements were based on feeding trials with reference diets (Akiyama *et al.*, 1991). The Table- 4 summarises the recommendations of various amino acids for penaeid shrimp (Akiyama *et al.*, 1991).

Table-5: Recommended essential amino acid levels in the diet of penaeid shrimps (Akiyama *et al.*, 1991)

Amino acid	% of protein
Arginine	5.8
Histidine	2.1
Isoleucine	3.4
Leucine	5.4
Lysine	5.3
Methionine	2.4
Methionine + cystine	3.6
Phenylalanine	4.0
Phenylalanine+ tyrosine	7.1
Threonine	3.6
Tryptophan	0.8
Valine	4.0

Recently, various methods have been used to overcome the problem in shrimps. A study using microencapsulated L-arginine indicated that a level of 2.5 g /100 g diet 5.5 g / 100 g protein is required to achieve optimal growth for juvenile *Penaeus monodon* (Chen *et al.*, 1992a, b). By adjusting dietary pH to neutrality and increasing meal frequency to 5 times per day, Liou and Yang (1994) successfully incorporated crystalline methionine and other amino acids in the diet of juvenile *Penaeus monodon* and estimated the methionine cystine requirement to be 1.4 g / 100 g diet 4.0 g / 100 g protein. Millamena *et al.* (1996) found the requirements of *Penaeus monodon* for valine to be 3.75 g / 100 g protein.

2.4.3.3. Protein-Energy interactions

Protein requirement can be overestimated by a low dietary energy level that induces a high catabolism of amino acids to derive required energy, and also if proteins of low digestibility or biological value are used. The ability of crustaceans to utilize carbohydrates and lipids as energy sources sparing the valuable protein for

growth varies with species, size, physiological state, protein quality and digestibility, and the level of non-protein energy in the diet. Since the available information on these lines is limited for commercial crustacean species, and the hierarchy of information on energy digestibilities measured in fish can be applied to crustaceans (Cuzon and Guillaume, 1997).

2.4.4. Dietary lipids and fatty acids

Dietary lipid is vital to satisfy the fatty acid (FAs) requirements, particularly the essential fatty acids (EFAs) which are not synthesized *de novo* by the organism, but necessary for the synthesis of eicosanoids and for the maintenance of structural and functional integrity of biomembranes, in the form of polar memebrane lipids (phospholipids and glycolipids). Lipids also serve as a carrier during intestinal absorption of liposoluble vitamins and carotenoids, and when complexed with protein functions as carrier of chlolesterol and triglycerides in hemolymph. And finally considered as a source of energy, and in the intermediary metabolic pathways, the *de novo* lipid synthesis is very important as a mechanism of energy storage by the conversion of dietary excess of carbohydrates and proteins into neutral storage lipids (triglycerides) (Lehninger *et al.* 1991). Apart form these, dietary lipids, being an energy source, can spare the expensive protein without affecting the growth rate (D' Abramo *et al.*, 1997).

2.4.4.1. Dietary lipid requirement

The dietary level of lipid is influenced by a variety of factors like, the quality and quantity of protein and other energy sources in the diet and the quality (EFA profile) of the lipid source. Generally, the optimal dietary lipid requirement for crustaceans range from 5 to 8 % (D'Abramo *et al.*, 1997). The Table-6 shows the lipid levels recommended for the best growth response in various crustaceans.

Table-6: Optimal dietary lipid levels promoting best growth response in various crustaceans

Species	Lipid source	Best response level	Author(s)
Penaeus japonicus	Soybean oil, Pollock liver oil, short neck clam oil	8 % short neck clam oil alone	Kanazawa *et al.* (1977a)
Penaeus japonicus	Pollock liver oil and soybean oil (1:1)	6 %	Deshimaru *et al.* (1979)
Penaeus indicus	Marine and plant oils alone and in combinations	6-10%	Chandge and Paulraj (1990)
Homarus americanus	Cod liver oil	5 %	Castell and Covey (1976)
Macrobrachium roesnbergii	Cod liver oil, corn oil (2:1)	6 %	Sheen and D'Abramo (1991)
Procambrus clarkii	Menhaden oil	6-9 %, beyond 9% cause growth reduction	Davis and Robinson (1986)

In the formulated feed fed to juvenile *Scylla serrata,* Sheen and Wu (1999) recommended dietary levels of 5.3 to 13.8% lipid as a mixture of cod liver oil and corn oil at 2:1 (w/w) ratio.

2.4.4.4. Lipid sources

As given in Tables 6 and 7 various lipid sources like, marine oils (cod liver oil, menhaden oil, pollock liver oil, shark liver oil, short-neck clam oil, squid oil, shrimp head oil etc.), terrestrial animal fat (pork lard, beef lard etc.) and plant oils (corn oil, sunflower oil, soybean oil, safflower oil, linseed oil, ground nut oil etc.) in single or in different combinations were tried in different crustaceans (Castell and Covey, 1976;

Kanazawa *et al.* 1977a; Deshimaru *et al.* 1979; Davis and Robinson, 1986; Chandge and Paulraj, 1990; Sheen and Wu, 1999; Wen *et al.*, 2002; Xu, 1994).

Information on the suitability of various lipid sources for *S. serrata* is yet to be made available, and is a requirement for the development of commercially viable and nutritionally adequate formulated feeds.

Table-7: Efficacy of different lipid sources in the diets of various crustaceans

Author(s)	Species	Treatment	Conclusions
Colvin (1976)	*Penaeus indicus*	Diets containing either sunflower, linseed, soybean, or groundnut oil at a 5% level	No significant difference in growth after 35 day feeding trial
Kanazawa *et al.*,(1977a)	*P. japonicus*	Powdered pollack residual oil, liquid pollack residual oil, short-necked clam lipid and soybean oil at 8% inn diets	Marine oils superior to soybean oil when supplemented individually. Mixture of marine and vegetable oils yielded the best results
Deshimaru *et al,*(1979)	*P. japonicus*	Mixture of pollack liver oil and soybean oil provided at 6 % levels in diet	Ratio between 3:1 and 1:1 yielded better growth and feed efficiency

2.4.4.6. Cholesterol requirements

Crustaceans are incapable of synthesizing sterols *de-novo* (Teshima and Kanazawa, 1971; Teshima, 1972), and since cholesterol forms an important constituent of cells and a metabolic precursor of a number of steroid hormones like ecdysteroids (Teshima, 1972), dietary supplementation of cholesterol is considered to be essential for good growth and survival of crustaceans. Table-8 summarises the dietary cholesterol requirements of various crustaceans.

Table-8: Optimal dietary cholesterol levels promoting best growth rate in various crustaceans

Species	Best response level	Author(s)
Penaeus japonicus	0.5 %	Kanazawa *et al.* (1971)
Penaeus japonicus	2.1 %	Deshimaru and Kuroki (1974b)
Penaeus monodon	0.5 %	Chen (1993)
Carcinus maenas	1.5 %	Ponat and Adelung (1983)
Homarus americanus	0.19-0.59 %	D'Abramo *et al.* (1984)
Homarus americanus	0.25-0.5 %	Kean *et al.* (1985)
Penaeus indicus	0.5 %	Chandge and Paulraj (1996)

Sheen and Wu (2000) reported that the optimal level of dietary cholesterol for growth and survival of juvenile *Scylla serrata* is approximately 0.51 %.

2.4.4.7. Phospholipid requirements

The dietary phopholipid requirements of different crustaceans are summarized in Table-9.

Table-9: Dietary phospholipid requirements of various crustaceans

Species	Source of phospholipid and levels	Response	Author(s)
Penaeus japonicus	Soy lecithin -24% PC, 30% PE, 18% PI	3 %	Teshima *et al.* (1986 a,b)
Penaeus monodon	Soy lecithin	2 %	Piedad-Pascual (1986)
Penaeus indicus	Mixture of soy lecithin, cod liver oil and soybean oil	10 %	Chandge and Paulraj (1995)
Penaeus chinensis	Soy lecithin, 42% PC, 20% PI, 6% PE, 4% LPC	No significant effect, 0-6%	Kanazawa (1993)

Penaeus stylirostris	Soy lecithin	1.5 %	Bray *et al.* (1989)
Penaeus stylirostris	Soy lecithin, 42% PC, 20% PI, 6% PE, 4% LPC	2 %	Kanazawa (1993)
Penaeus penicillatus	Soy PC, mixture of 80% PC and 20% LPC	1.2 %	Chen and Jenn (1991)
Homarus juvenile	Soy lecithin	7.5 %	Conklin *et al.* (1980)
Homarus juvenile	Refined soy lecithin (95 % PC)	2 %	D'Abramo *et al.* (1981)
Homarus juvenile	Soy lecithin or crab phospholipids	No significant effect, 0-6%	Kean *et al.* (1985)

Phospholipid deficiency in *Homarus* juveniles resulted in moult death syndrome causing a lower survival rate (Conklin *et al.*, 1980). Teshima *et al.* (1986a,b,c) reported that in *Penaeus japonicus*, the growth rate and feed conversion efficiency (FCE) were significantly lower when fed a phospholipid deficient diet than a diet containing 3% phospholipids. The growth promoting effect of phospholipid is influenced by the quality of the dietary protein (Bray *et al.*, 1989; Jenn, 1989). The percentage weight gain in *Penaeus monodon* was found to increase when the phospholipid level was raised from 0 to 2 %, irrespective of the lipid source (Piedad-Pascual, 1986).

2.4.4.2. Fatty acid requirements

Growth of crustaceans is affected not only by the quantity of dietary lipids but also by the quality of lipid compounds, which is a function of its fatty acid profile, particularly the unsaturated fatty acids and their degree of unsaturation (D'Abramo *et al.*, 1997). The essential fatty acids function as an important source of energy and maintain the functional integrity of bio-membranes (Lehninger *et al.* 1991). The combinations of n-3 and/ or n-6 essential fatty acids (EFAs) such as linoleic acid (LA, 18:2n-6), linolenic acid (LNA, 18:3n-3), arachidonic acid (AA, 20:4n-6), eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3) are required in the diets of all marine animals including important cultured

crustaceans and fishes (Castell *et al.*, 1972; Kanazawa and Teshima, 1977, 1979; Kanazawa *et al.*, 1978; Sargent *et al.*, 1989; D' Abramo & Sheen, 1993; Xu *et al.*, 1994; D'Abramo *et al.*, 1997; Merican and Shim, 1995). Out of these 18:2n-6, 18:3n-3 and 20:4n-6 are known as polyunsaturated fatty acids (PUFAs), and 20:5n-3 and 22:6n-3 are known as highly unsaturated fatty acids (HUFAs) depending on the carbon chain length and the extent of unsaturation (D'Abramo *et al.*, 1997).

The moulting frequency of crustaceans is influenced by the synthesis of hormone-like components such as eicosanoids. Eicosanoids are derived form FAs such as 20:4n-6, 20:5n-3, and 22:6n-3 (Subramoniam, 2000). Castell and Covey (1976) reported that American lobster, *Homarus americanus* fed a lipid-free diet had a lower moulting frequency. Sheen and Wu (1999) also found that the moulting frequency of juvenile *S. serrata* fed diets supplemented with lipid was higher than those fed a lipid-free diet. Crustaceans injected with with prostaglandin E2, a type of eicosanoid, displayed a shorter inter-moult period than did the control animals (D'Abramo,1997). From the above studies it was clearly established that the FAs, viz. 20:4n-6, 20:5n-3, and 22:6n-3 are important dietary essentials being the precursors for the biosynthesis of eicosanoids, which promote moulting and growth in crustaceans.

In mammals, linoleic acid (18:2n-6) is an important essential fatty acid (EFA) and is bio-converted *in vivo* to the physiologically more active, arachidonic acid (20:4n-6), which is an important precursor for the eicosanoid metabolism (Lehninger *et al.*, 1991). However, studies on the nutritional requirements of marine fish and shrimp have shown that fatty acids of the n-3 family have greater EFA value for these species than fatty acids of the n-6 family (Castell and Covey, 1976; Castell *et al.*, 1972; Kanazawa *et al.*, 1979a,b; Castell, 1982; Xu *et al.*, 1993).

Kanazawa and Teshima (1977,1979a,b) demonstrated that several species of marine crustaceans exhibited only limited ability to elongate and desaturate 18:C polyunsaturated fatty acids (PUFA) of the n-3 and n-6 series to the longer-chained, more highly unsaturated forms (HUFAs) necessitating dietary supplementation at adequate levels for normal metabolism. In *Penaeus chinensis* there is evidence of

C:18 FAs getting elongated to 20:2n-6 and 20:3n-3, but not apparently found to elongate and desaturate to 22:6n-3 and 20:5n-3 (Xu *et al.* 1993). Also 20:4n-6 is found to be superior to 8: 2n6 and 18:3n3 in giving better survival. The larvae of *Scylla serrata*, when fed with enriched and un-enriched live feeds, were found incapable to bio-convert the C:18 PUFAs to HUFAs (Suprayudi *et al.*, 2003).

The studies in *Penaeus japonicus,* (Kanazawa and Teshima, 1977, 1979 a,b), *Penaeus indicus* (Read, 1981; Chandge and Paulraj, 1990), and *Palaemon serratus* (Martin, 1980), have shown that 18:3n-3 had greater EFA value than 18:2n-6. In *M. japonicus* though linoleic acid *(*18:2n-6*)* and linolenic acid (18:3n-3) are found essential for the growth (Kanazawa *et al.*, 1977b), longer-chain n-3 highly unsaturated fatty acids (HUFAs), such as 20:5n-3 (Kanazawa *et al.*, 1978) and 22:6n-3 (Kanazawa *et al.*, 1979a,b) had greater EFA value than 18:2n-6 or 18:3n-3. Similar results have also been reported by Read (1981) in *Penaeus indicus.* Xu *et al.* (1993) demonstrated the order of nutritional value of the purified fatty acids as 22:6n-3 > 20:4n-6 > 18:3n-3 > 18:2n-6 for the Chinese prawn *Penaeus chinensis,* when added individually at 1 % of the diet.

Studies on the swimming crab *Portunus trituberculatus* showed that larvae fed rotifers cultured on freshwater chlorella *(Chlorella vulgaris)* or fortified with corn oil that lacked n-3 HUFA rarely survived to the first crab stage indicating that 20:5n-3 and 22:6n-3 are more essential for the swimming crab than 18:2n-6 and 18:3n-3 (Hamasaki *et al.*, 1998; Takeuchi *et al.*, 1999a). Moreover, Takeuchi *et al.* (1999b) described the requirement of EPA and DHA for larval development, where EPA is effective in maintaining survival while DHA plays an important role in accelerating the moulting rate and produces a wider carapace width in swimming crab larvae. However, a study on *Scylla paramamosain* showed that larvae of this crab can reach the first crab stage when fed newly hatched *Artemia* nauplii that contain a high amount of 18:3n-3 with a small amount of 20:5n-3, but deficient in 22:6n-3 (Kobayashi *et al.*, 2000). Suprayudi *et al.* (2004) demonstrated DHA as nutritionally superior to that of EPA, LA and LNA in terms of a higher survival, shorter intermoult period and broader carapace width at the first crab stage in *S. serrata.* The studies

of Hamasaki *et al.* (2002) and Suprayudi *et al.* (2002a,b) showed that the EFA contained in newly hatched *Artemia* did not match the requirement of mud crab larvae and led to a low survival rate at the first crab stage. During rotifer feeding, it was found that mud crab larvae required enrichment with n-3 HUFAs for better survival and shorter intermolt period (Hamasaki *et al.*, 2002; Suprayudi *et al.,* 2003). Investigations in juvenile *Scylla serrata* fed formulated diets supplemented with purified fatty acid methyl esters, showed that a dietary source of 22:6n-3, 20:4n-6 and 18:3n-3 is required for normal growth (Sheen and Wu, 2003).

2.4.4.3. Fatty acid metabolism

Crustaceans have little ability to synthesize either linolenic or linoleic families of fatty acids *de novo* (Kayama *et al.,* 1980). The body tissue of marine crustaceans generally tends to contain proportionately higher levels of PUFA and HUFA of the linolenic family than that of freshwater crustaceans, which are derived through the food chain (Castell, 1983). Similarly, freshwater crustaceans characteristically have higher levels of fatty acids of the linoleic family in their body tissue (Chanmugam *et al.*, 1983). The linolenic family has been observed to have the greatest EFA value for marine animals (Castell and Boghen 1979).

Studies with ^{14}C incorporated acetate in *Penaeus japonicus* showed that the metabolic activity is almost exclusively associated with the saturated (16:0, 18:0) and monounsaturated (16:1, 18:1n-9, 20:1n-9) fatty acids and less than 2% of the total activity was found in each of the 18:2n6, 18:3n3, 20:5n3 and 22:6n3 fatty acids (Kanazawa and Teshima, 1977). Further, Kanazawa *et al.* (1979a,b) have found that *M. japonicus* was able to convert palmitic acid (16:0) to other saturated and mono-saturated fatty acids. However, little or no activity originating from labeled palmitic acid was found in linoleic acid (18:2n6), linolenic acid (18:3n3), eicosapentaenoic acid (20:5n3), and docosahexaenoic acid (22:6n3). These early studies suggested that dietary sources of PUFA and HUFA might be essential for the growth of crustaceans due to the lack of biosynthetic ability, evidenced in *P. indicus* (Read, 1981; Colvin, 1976b), *Penaeus setiferus, Penaeus aztecus and Penaeus durarum*

(Bottino *et al.*, 1980). Later Kanazawa *et al.* (1979b) established that *Penaeus japonicus* had some ability to convert [^{14}C] linolenic acid (18:3n3) to 20:5n3 and 22:6n3.

Dall (1981) found that digestion and absorption of ^{14}C tripalmitate in the Norwegian lobster, *Nephrops norvegicus* was complete in 8 to 12 hours after ingestion. The apparent digestibilities of a variety of lipids (oils, palmitic acid, oleic acid, tripalmitin, and egg lecithin) exceeded 80% when included at a level of 8 % in diets fed to *Marsupenaeus japonicus* (Teshima and Kanazawa, 1983). Triacylglycerols in full fat soya, fish, squid, squid liver, and fish liver meals were highly digested (96.1-99.8%) when these ingredients were included in diets fed to juvenile *Penaeus monodon* (Merican and Shim 1995). The apparent digestibilities of fatty acids are somewhat different relative to the triacylglycerol source. Some major trends suggested by the studies of Merican and Shim (1994) with *Penaeus monodon* are: that the majority of fatty acids had apparent digestibility coefficients that exceeded 90%, digestibility of saturated fatty acids are inversely related to chain length, where as a direct relationship was found with mono-enoic fatty acids, and the digestibility of individual fatty acids was influenced by the presence of other fatty acids.

2.4.4.5. Essential fatty acid supplementation

The effect of dietary supplementation of essential fatty acids and their relative nutritive value were established for many crustacean species such as *Penaeus japonicus* (Guary *et al.*, 1976, Kanazawa *et al.*, 1977b, 1979a, 1979d), *Penaeus aztecus* (Shewbart and Miles, 1973), *Penaeus indicus* (Read, 1981), *Penaeus chinensis* (Kanazawa *et al.*, 1977b; Xu *et al*, 1993, 1994), *Penaeus stylirostris* (Fenucci *et al.*, 1981), *Palaemon serratus* (Martin, 1980) and *Macrobrachium rosenbergii* (D' Abramo and Sheen, 1993). A collective examination of results in the case of *Macrobrachium rosenbergii* suggests that the best response would be achieved at a high ratio of 18:n6 and 18:n3 PUFAS (Reigh and Stickney, 1989; Teshima *et al.*, 1992). Table-10 summarises the dietary fatty acid supplementation in shrimps and prawn

In their study to establish optimal diet for *Carcinus maenas*, Ponat and Adelung (1980) reported that, cod liver oil provided a good source of lipid in the formulated gel feeds.

Supplementation of plant lipid sources in the artificial diets of *Penaeus indicus* resulted in elevated tissue depot level of linoleic acid (18:2n-6), suggesting limited ability of the shrimp to bio-convert this fatty acid in to longer chain unsaturates (Colvin, 1976a,b). The study also suggested the need for a balanced n3/n6 ratio in the diet.

Table-10: Dietary supplementation of fatty acids in various crustaceans

Species	Treatment	Conclusions	Author(s)
Penaeus japonicus	Supplementation of 18:2n6 and 18:3n3 with oleic acid, pollack residual liver oil, soybean oil, or short-necked clam lipids in diets.	Weight gain improved with 1% inclusion of either 18:2n6 or 18:3n3 in the diets	Kanazawa *et al.* (1977b; 1979a; 1979d)
Juveniles of *Penaeus aztecus*	Supplementation of 0.5 to 5 % linolenic acid in commercial shrimp feed	Best growth response recorded with 2% linolenic acid. Growth declined beyond 2% levels.	Shewbart and Miles (1973)
Penaeus indicus	Addition of either 18:2n6 or 18:3n3 or a mixture (1:1) to diets	1% addition of either18:2n6 or 18:3n3, improved growth and survival.	Read (1981)
Penaeus japonicus	Relative nutritive value of 18:3n3 vs. 18: 2n6	Diets containing high levels of 18:3n3 was found better than that of equivalent levels of 18: 2n6	Guary *et al.* (1976)

Penaeus chinensis	Relative nutritive value of 18:3n3 vs. 18: 2n6	" "	Xu *et al.* (1994a)
Macrobrachium rosenbergii	" "	At 1% dietary level 18: 2n6 found superior to 18:3n3 for postlarval growth	Reigh and Stickney (1989)

The studies carried on the n3-n6 interactions and ratios in *Penaeus stylirostris* (Fenucci *et al.*, 1981), *Palaemon serratus* (Martin, 1980) and *Macrobrachium rosenbergii* (Teshima *et al.*, 1992) are summarized and presented in Table-11.

Table-11 : Dietary n3-n6 interactions in various crustaceans

Species	Treatment	Deductions	Author(s)
Penaeus stylirostris	I8:3n-3 to 18:2- n6 dietary ratio	Recommended 1.18 :1.00 to achieve the best growth in juveniles	Fenucci *et al.* (1981)
Palaemon serratus	proportions of 18:2n6 and I8:3n-3 by varying the relative amounts of soybean oil and linseed oil	Best growth achieved with 18:2n6/18:3n3 ratio of 2.2	Martin (1980)
Macrobrachium rosenbergii	18:2n6 to 18:3n3 ratio	highest weight gains achieved at 12:1 ratio	Teshima *et al.* (1992)

The combination of a HUFA rich marine oil source with PUFA rich plant oil source was found to be superior for growth performance in *Penaeus indicus* (Chandge and Paulraj, 1995), *Macrobrachium rosenbergii* (Sheen and D'Abramo, 1991), *Penaeus monodon* (Sheen *et al.* 1994), *Scylla serrata* (Sheen and Wu, 1999), Chinese mitten crab, *Eriocheir sinensis* (Wen *et al.*, 2002) and as well as for reproductive performance in *Macrobrachium rosenbergii* (Hien *et al.*, unpublished). The performance may be attributed to the improved balance of n-3 and n-6 EFAs of the lipid combination than available in individual lipid sources.

2.4. 5. Minerals

With the exception of osmotic regulation, biochemical functions of minerals in aquatic animals appear to be similar to those in terrestrial animals, such as components of hard-tissue matrices, soft tissues, metalloproteins, and as cofactors or activators of a variety of enzymes, and the more soluble minerals function in osmoregulation and the maintenance of acid-base balance and membrane potentials (Lovell, 1989). Unlike terrestrial animals which primarily derive minerals from dietary sources, aquatic animals are able to utilize minerals dissolved in water to some extent. Consequently the determination of quantitative dietary requirement using graded levels of the element of interest in diet is difficult (Lall, 1989).

In spite of this, various authors have worked on mineral requirements of crustaceans, particularly shrimps and lobsters, using semi-purified and practical feeds. Deshimaru and Kuroki (1974a) using semi-purified diets, found that the total mineral content as high as 19.5% produced the best growth response in *Penaeus japonicus. Litopenaeus vannamei* reared in cages fixed in natural ponds, required dietary mineral supplementation in practical feeds to get the best growth performance (Castille and Lawrence, 1989). Dietary mineral requirements reported for various crustaceans including shrimps and lobsters are summarized in Table-12.

From the Table-1 it is evident that in spite of being in the aquatic medium, particularly in brackishwater and seawater media, which are rich in minerals, there exists a dietary requirement for mineral nutrients by crustaceans (Davis *et al.*, 1993c). The information on Ca:P ratio is vital since there exists an inverse relation between P and Ca in the diet (Gallagher *et al.*, 1978; Davis *et al.*, 1993c) though a poor correlation is found in *Penaeus vannamei* (Davis *et al.*, 1993c). Watanabe *et al.* (1988) proved an inverse relationship between tricalcium phosphate and zinc in ingredients like fish meal. Similarly the availabilty of P and Zn are affected by factors such as bound phytate in the practical diets making these minerals biologically unavailable (Davis *et al.*, 1993b; Gatlin and Philips, 1989; Gatlin and Wilson, 1984a,b).

Due to the low bioavailability or deficiency of minerals, most practical diets will require supplementation of a highly available phosphorus source along with trace minerals, such as copper, manganese, zinc and selenium. At the same time excessive mineral supplementation can lead to phosphorus enrichment in the aquatic system, causing eutrophication (Davis and Lawrence, 1997).

Table-12: Recommended dietary mineral dosages for shrimps and lobster

Mineral	Species	Recommendation (Dietary %)	Author(s)
Ca	*P. japonicus*	Dispensable	Deshimaru and Yone (1978a)
	" "	1.2 %	Kitabayashi *et al.* (1971)
	" "	1-2 %	Kanazawa *et al.* (1984)
	P. vannamei	Dispensable	Davis *et al.* (1993c)
P	*P. japonicus*	2 %	Deshimaru and Yone (1978)
	" "	1 %	Kitabayashi *et al* (1971)
	" "	1-2 %	Kanazawa *et al.* (1984)
	" "	For 1 % Ca -0.75 %	Civera (1989)
	P. vannamei	0.34% with 0.03% Ca 0.5-1.0% with 1% Ca 1.0-2.0% with 2% Ca	Davis *et al.* (1993c)
Ca:P ratio	Juvenile *Homarus americanus*	0.56:1.1	Gallagher *et al.* (1978)
	Adult *Homarus americanus*	1:1	Gallagher *et al.* (1982)
	P. japonicus	1:1	Kanazawa *et al.* (1984)
	" "	1.24:1.04	Kitabayashi *et al.* (1971)
	P. californiensis	2.06:1.0	Huner and Colvin (1977)
	P. vannamei	Poor correlation	Davis *et al.* (1993c)

K	*P. japonicus*	1 %	Deshimaru and Yone (1978)
	" "	0.9%	Kanazawa *et al.* (1984)
Mg	*P. japonicus*	Dispensable	Deshimaru and Yone (1978)
		0.3%	Kanazawa *et al.* (1984)
Cu	*P. japonicus*	Dispensable	Kanazawa *et al.* (1984)
	P. orientalis	5.3 mg/100g diet	Liu *et al.* (1990)
	P. vannamei	1.6-3.2 mg/100g diet	Davis *et al.* (1993a)
Fe	*P. japonicus*	Dispensable	Deshimaru and Yone (1978)
	" "	Dispensable	Kanazawa *et al.* (1984)
	P. vannamei	Dispensable	Davis *et al.* (1993b)
Mn	*P. japonicus*	Dispensable	Kanazawa *et al.* (1984)
Se	*P. vannamei*	0.02-0.04 mg/100g diet	Davis (1990)
Zn	*P. vannamei*	1.5-20 mg/100g diet	Davis *et al.* (1993b)

The requirement of minerals by crustaceans vary with the culture environment, life stage, moult stage etc. and the review of literature reveals that scanty information is available currently on very few species, like shrimps and lobster, with lots of contradictions. The bioavailabilty of minerals in the feedstuffs and mineral supplements to crustaceans is yet to be understood thoroughly, and the information is vital to budget the mineral supplementation in practical feeds, reduce negative interactions between various mineral nutrients, optimize the feed performance and cost, and reduce feed derived waste output from farms.

2. 4.6. Vitamins

Vitamins are dietary essential organic compounds for crustaceans, required in trace quantities, and their absence or deficiency can limit the metabolic processes of

the organisms. Most of the vitamins function as co-enzymes of various enzymes in biological systems (Conklin, 1997). Like mineral requirements, information on vitamin requirement of crustaceans is very scarce and the available information is incomplete (Conklin, 1997). In low stocking density culture systems, vitamin requirements are met by the natural food. But in semi-intensive and intensive culture systems, they are dietary essential micronutrients critically affecting the feed performance (Biddle, 1977; New, 1976). Most of the work relating to vitamin nutrition in crustaceans is focused on shrimps to recommend dosages for commercial feeds. Apart from shrimps there exists only very few studies on vitamin nutrition in decapod crustaceans such as crabs (Ponat and Adelung, 1980; 1983) crayfish (Brown, 1995; D'Abramo and Robinson, 1989) freshwater prawn (D'Abramo and Sheen, 1994) and lobsters (Conklin, 1995; Kanazawa, 1994). The dosages recommended in commercial feeds for different species of shrimps are summarised in Table-13.

Table-13: Recommended dosage of vitamins in commercial feeds for various species of shrimps and prawns

Species	Vitamin	Recommendation mg/kg (specified otherwise)	Author(s)
Water soluble vitamins			
Penaeus japonicus	Thiamine	60	Deshimaru and Kuroki (1979)
Penaeus japonicus	" "	50-60	Akiyama *et al.*(1992)
Penaeus monodon	" "	15 – 60	Chen *et al.* (1991)
Penaeus japonicus	Riboflavin	40	Akiyama *et al.* (1992)
Penaeus monodon	"	20	Chen and Hwang (1992)
Penaeus monodon	"	Dispensable	Catacutan and De la Cruz (1989)
Macrobrachium rosenbergii	"	Dispensable	Heinen (1984)

Penaeus japonicus	Niacin	200	Akiyama *et al.*(1992)
Penaeus monodon	"	10	Shiau and Suen (1994)
Penaeus japonicus	Vitamin B_6	60	Deshimaru and Kuroki (1979)
Penaeus japonicus	"	50	Akiyama *et al.*1(992)
Penaeus rosenbergii	"	Essential, but quantity not established	Heinen (1984)
Penaeus japonicus	Biotin	10	Akiyama *et al.* (1992)
Penaeus japonicus	Folic acid	1	Akiyama *et al.* (1992)
P. monodon	"	2-8	Chen (1993)
Penaeus japonicus	Vitamin B_{12}	0.1	Akiyama *et al.*(1992)
Penaeus monodon	"	0.2	Shiau and Lung (1994)
Penaeus japonicus	Choline	600	Kanazawa *et al.* (1976)
Penaeus japonicus	"	Dispensable	Deshimaru and Kuroki (1979)
Penaeus japonicus	"	400	Akiyama *et al.*,(1992)
Penaeus japonicus	*myo*-Inositol	400	Kanazawa *et al.* (1976); Deshimaru and Kuroki (1979)
Penaeus japonicus	"	300	Akiyama *et al.*,(1992)

Penaeus monodon	L-ascorbic acid Stable Vitamin C, sulfates and phosphates	40- 157	Shiau and Hsu (1994)
Penaeus japonicus, Penaeus vannamei, Penaeus monodon Macrobrachium. rosenbergii	Stable vitamin C	100-200	Shigueno and Itoh (1988); He and Lawrence (1993); Chen and Chang (1994), D'Abramo *et al.* (1994)
Fat soluble vitamins			
Penaeus vannamei	Vitamin A	4800 IU/kg	He *et al.* (1992)
Penaeus japonicus	"	10000 IU/kg	Akiyama *et al.* (1992)
Penaeus japonicus	Vitamin E	300	Akiyama *et al.* (1992)
Penaeus vannamei	"	100	He and Lawrence (1993)
Penaeus vannamei	Vitamin D	8000 IU/kg	He *et al.* (1992)
Penaeus japonicus	"	5000 IU/kg	Akiyama *et al.* (1992)
Penaeus monodon	"	40 IU/kg	Hwang (1994)
Penaeus japonicus	Vitamin K	5	Akiyama *et al.* (1992)
Penaeus vannamei	"	40	He *et al.* (1992)
Penaeus monodon	"	35	Shiau and Liu (1994)

Excess of vitamins like riboflavin, niacin, and vitamin B_6, A, D and K in levels in crustacean feeds can negatively affect growth (Cobun, 1994; Conklin, 1997; McDowell, 1989; Shiau and Suen, 1994). Therefore a through revision of vitamins in diets with modification of dosages is very much essential. Though required in trace quantities, vitamins are costly ingredients and have substantial control over the feed production cost when added. Hence optimized dosages are very much essential for the profit maximization of crustacean production systems.

CHAPTER III
Materials and Methods

3. MATERIALS AND METHODS

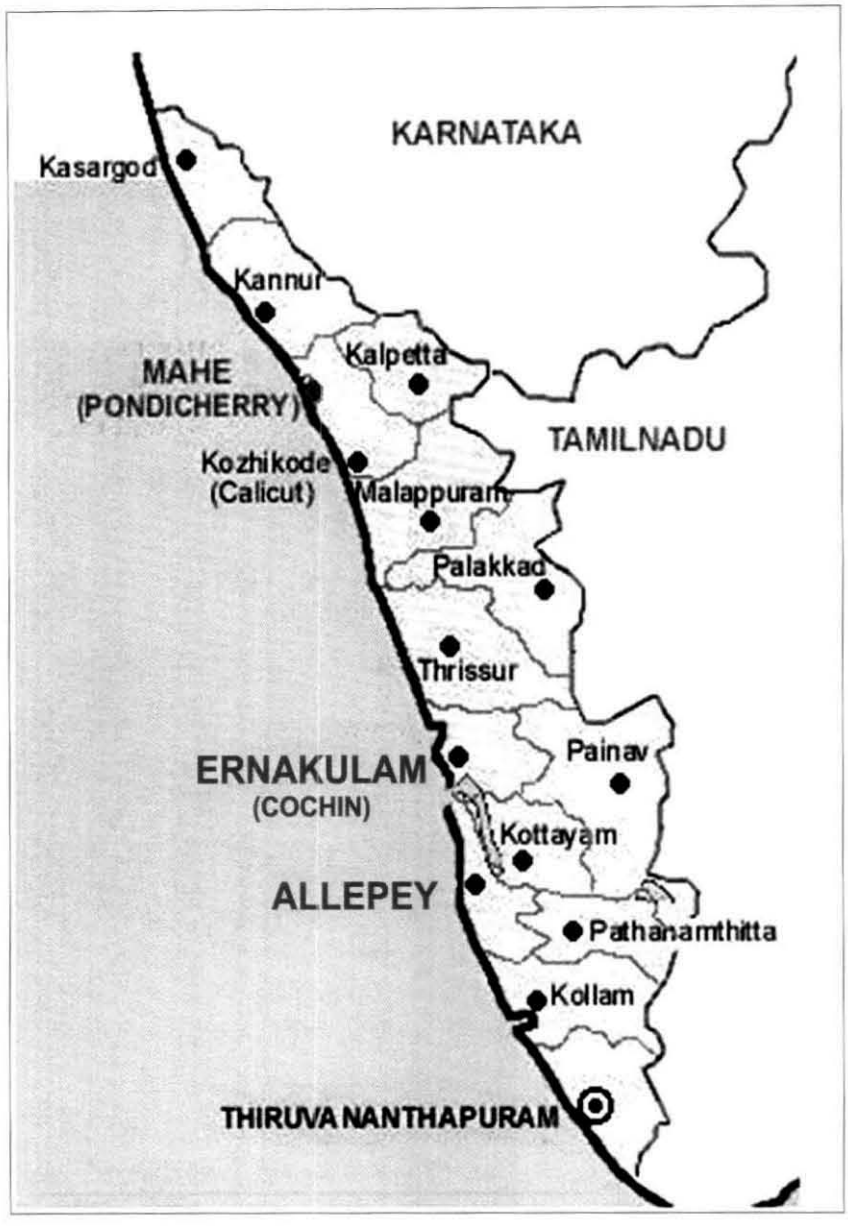
3.1. Survey of farming practices, feeds and feed management in mud crab farms

The mud crab farming along the Kerala coast begins soon after the withdrawal of the south-west monsoon during September-October, when the salinity in the backwaters and ponds stabilizes above 10 ppt and water temperature reaches 20-25 ° C, and extends up to May, till the onset of south-west monsoon. The farming activity sometimes is continued during the monsoon months (June-September) in few farms, which are located very close to the sea wherein salinity does not drop below 8 ppt. However, in such farms the crab yield is relatively low with duration required for growth and fattening will be more as the temperature and salinity are at their lowest levels during the monsoon. Though the mud crabs spawn throughout the year, the peak spawning season along the Kerala coast is during February-May. Numerous juvenile mud crabs of 25-50 g size occur in the backwaters during July-September, as the baby crabs migrate from the marine spawning grounds to the rich feeding grounds in the estuarine backwaters. They also enter in to the traditional prawn filtration fields and also contribute towards the productivity. These juvenile crabs form the seed source for the grow-out culture.

In addition to grow-out culture, fattening culture is also practiced. The availability of water crabs of above 250 g size for fattening culture is marginal from August to October, which marks the beginning of the season. But as the season advances, substantial numbers of water crabs are collected in large quantities.

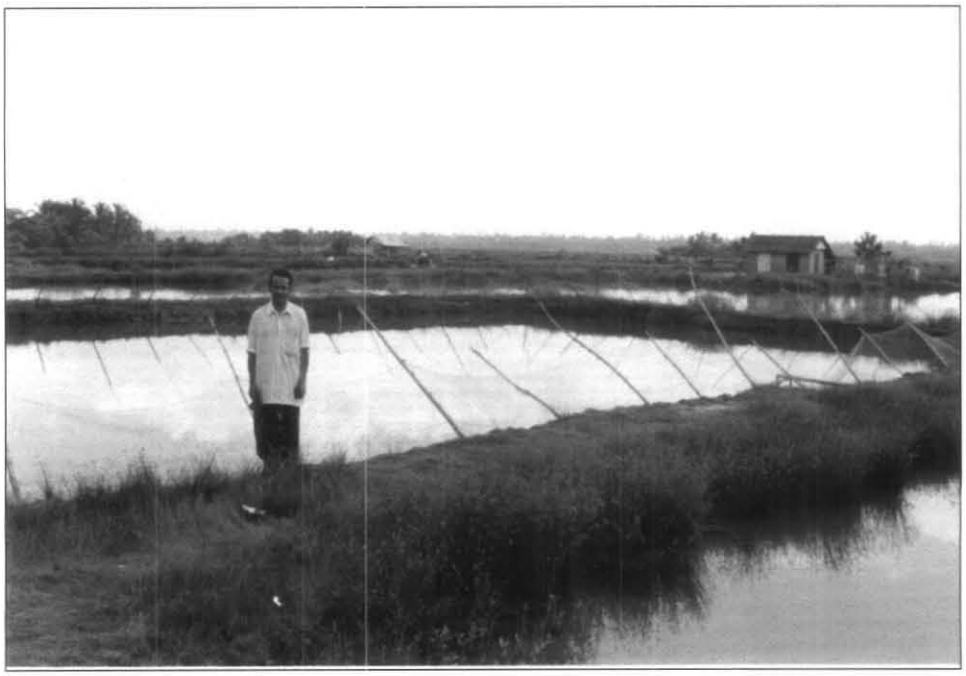
Extensive farm surveys were conducted in 28 mud crab farms in and around Ernakulam and Alleppey districts (Plate-1) from September 2000 to April 2001, based on frequent visits to the farms to gather information on the prevalent mud crab farming practices with special thrust on feeds and feed management.

PLATE-2

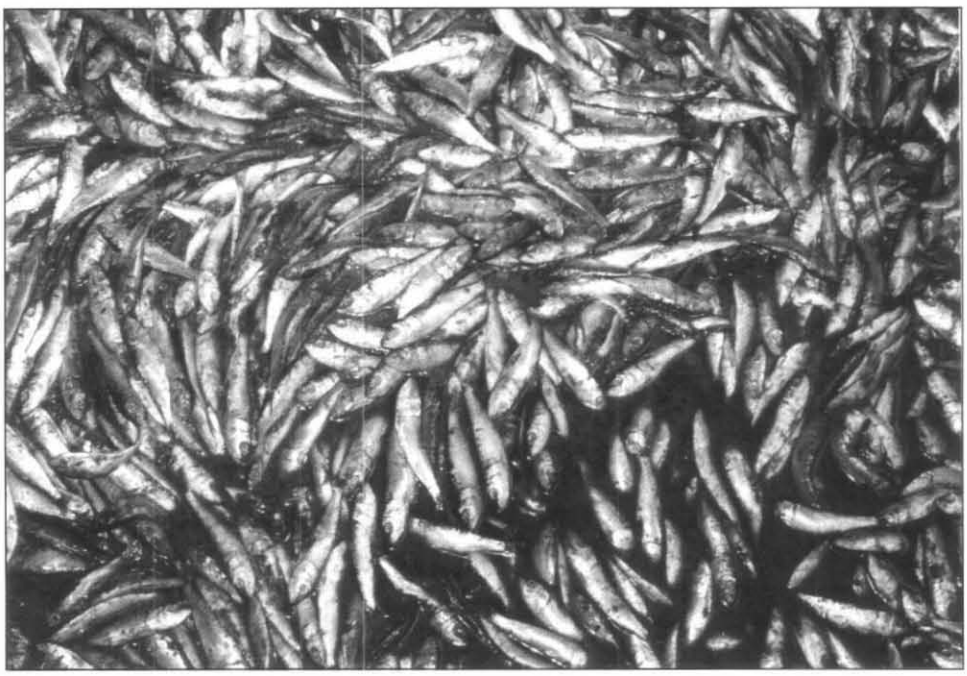


Survey of mud crab farming and feed management in Ernakulam and Allepey Districts in Kerala

PLATE-3



Mud crab farm located at Vypeen Island, off Cochin



Sardines stored in brine - widely used feed in mud crab culture

3.2. Efficacy of fresh and processed natural feeds

3.2.1. Collection transportation and acclimatization of juvenile crabs

Juvenile *Scylla serrata* were collected from the estuarine creek at Puthuvypeen, Narakkal and Ezhikkara located in Vypeen Island and also from the Kadamakkudy Island, off Cochin during October - November 2001. The physico-chemical parameters of the collection sites were viz., ?depth 3-8 m, salinity 22-29 ppt, temperature 23-27 °C, pH 7.8-8.5, DO 4.5-5.78 ppm and ammonia < 0.05 ppm.

The crabs were caught from the creeks by setting a number of ring net traps baited with freshly caught fishes such as *Tilapia* sp and *Glossogobius* spp. and also fresh sardines, on-board a plank-built canoe of ~ 4 m OAL. The fishing operation started around 1600 hrs and lasted till 2200 hrs. The traps were hung from a buoy and the length of the rope was set in such a way that the ring net trap was close to the bottom without touching the bottom. The crabs crawling at the bottom get attracted to the bait and get entangled in the net. The traps were taken out of water at 15-20 minutes intervals to collect the trapped crabs. The crabs were immobilized by tying the claws using cotton thread and packed in plastic bags with wet sponge placed on top to keep the crabs moist. Carapace width and body weight of the crabs were measured to the accuracy of 0.01 mm and 0.01 g using vernier calipers (Mitutoyo, Japan) and an electronic balance (Mettler, USA) respectively.

Sixty numbers of *Scylla serrata* juveniles of average body wt. 52.0 ±5.31g and carapace width 50.0 ±3.21 mm were acclimatized to the laboratory conditions for a period of one week, by stocking them individually in 50 litre deep blue coloured plastic troughs containing 40 litres of brackishwater (Table-14). The crabs were acclimatized to the physico-chemical water parameters, viz., salinity 28 ±1 ppt, temperature 28 ±4 °C, pH 8 ± 0.5 and DO > 4 ppm over a period of 10 days. The total ammonia in the water was found < 0.05 ppm.

The crab juveniles were fed a diet of live brackishwater black clams, *Villorita cyprinoides,* at *ad libitum* to induce moulting and 50 % of water was exchanged daily at 0700 hrs.

3.2.2. Experimental feeds

Eight different feed treatments were selected viz., fresh clam, frozen clam, dry clam, frozen fish, salted fish and, 3 combinations (SCF-1, 1: 2: 7, SCF- 2, 1: 4: 5 and SCF-3, 1: 5: 4) of frozen shrimp head (S), frozen clam(C) and frozen fish (F).

The live brackishwater clam *Villorita cyprinoides,* the most abundant clam species in Cochin backwaters, was collected from the brackishwater ponds of Krishi Vigyan Kendra of Central Marine Fisheries Research Institute, Vypeen Island, near Cochin. Using a specially designed galvanised iron netting-scoop operated on-board a FRP canoe of 3 m OAL, the clams were scooped along with the substratum every morning. Further, the clams were washed with water and stocked in 500 l circular FRP tanks containing sand filtered pond water for 8 hrs. During this period clams were found to egest the mud and fecal matter. To minimize the size variation and condition of the clams those with ~30 mm shell breadth were chosen for shucking the meat and fed to the crabs freshly.

The procedure mentioned above was also followed to collect the clam meat for frozen storage and dry storage. To prepare frozen stored clam, freshly shucked clam meat was packed in self-sealing food grade polyethylene bags and frozen at -20 °C. To prepare the dry clam meat freshly shucked clam meat was spread over an enameled metal plate and kept in a hot air oven and dried at 55 °C till the moisture content reached less than 8.0 % and stored in food grade polyethylene bottles in a dessicator at room temperature.

The fish selected for feeding was the Indian oil sardine, *Sardinella longiceps,* the most abundant pelagic fish in the coastal waters of Cochin, and the fish were procured from the Murikkumpadom Harbour at Vypeen Island. The fishes were

beheaded, degutted and washed with filtered brackishwater, packed in ≃ 250 g lots in food grade self-sealing polyethylene bags, and frozen at -20 °C

Another lot of sardines processed as above were stored in saturated brine solution having excess of common salt crystals in a 10 l capacity food grade polyethylene transparent bucket with a tight lid under room temperature.

Fresh heads of the Indian white shrimp, *Penaeus indicus* were procured from the peeling sheds at Najarakkal, Vypeen Island, packed in to ≃ 250 g lots in food grade self-sealing polyethylene bags and kept under frozen storage at -20 °C.

The frozen feed items were allowed to thaw for 10 minutes prior to weighing and feeding to the crabs. The fish stored in brine were washed in filtered pond water to remove the adhering salt, prior to weighing and feeding.

3.2.3 Experimental setup and feeding

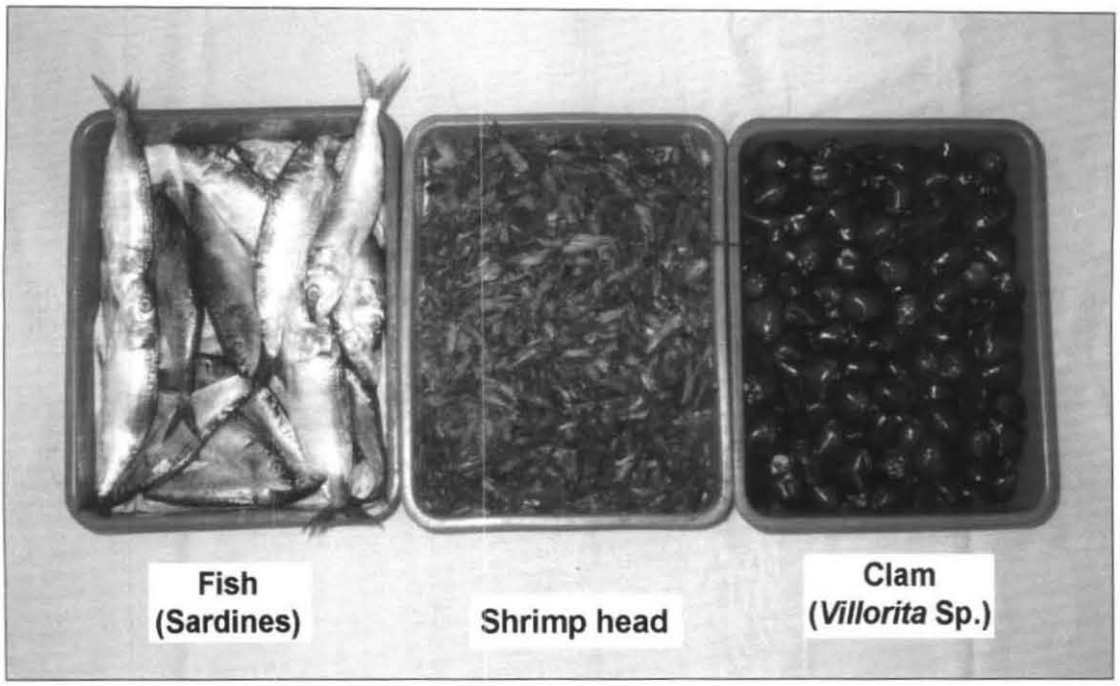
Out of the 60 juvenile crabs, 6 numbers in the post-moult stage were subjected to euthanasia by quick freezing at -20 °C and dried at 55 °C in a hot air oven and subjected to biochemical analyses as detailed in section 3.4.1. to know the initial body composition. Another batch of 48 juvenile crabs were selected on first moult - first select basis at early post-moult stage. Once the crab moulted, it was weighed and released back to a trough filled with fresh brackishwater. The feeding trials were commenced on the day following moulting. Uniformity in the size of animals was maintained (weight 89.0 ±4.28 g and carapace width 85.0 ±3.52 mm, Table-14).

Eight feed treatments, each having 6 replicates were set up. Based on observations on feed intake, the feeding level was adjusted to reduce wastage and ensure water quality. Except dry clam and salted fish all other feeds were offered at the rate of 7.5 % of body weight, whereas dry clam and salted fish were offered at 2.5 % and 5.0 % of body weight respectively.

Table-14: Details of experimental set up for the evaluation of fresh and processed feeds fed to juvenile *Scylla serrata*

Experimental duration: 54-120 days		
Biological parameters		
	Size at collection	Size at experimental stocking (1st moult)
Average Body wt. (g)	52.0 ±5.31	89.01 ±2.06
Average CW (mm)	50.0 ±3.21	85.07 ±1.87
Moult stage	Throughout one moult cycle	
Water quality parameters		
Salinity (ppt)	28 ±1	
Temperature (°C)	23.6 ±3.5	
DO (ppm)	>4.0	
pH	8.0 ±0.5	
Total NH_3 (ppm)	< 0.05	
Statistical parameters		
Randomized block design		
6 replicates per treatment with one crab per replicate		
Feed parameters		
Source	Fresh, frozen & dry clam meat, Frozen & salted fish (sardine) and Three combinations of shrimp head (S), frozen fish (F) and frozen clam (C), viz. SCF 1 - 10: 20 : 70 SCF 2 - 10: 40 : 50 SCF 3 - 10: 50 : 40	
Feed ration	Dry clam: 2.5%, Salted fish: 5.0% All fresh and frozen feed : 7.5% of body wt.	
Feeding schedule	Single ration at 1700 hrs, left to feed till it's removed next morning at 0700 hrs.	

PLATE-4



Feedstuffs used in the evaluation of fresh, processed and combination diets for juvenile *Scylla serrata*

PLATE-5

Experimental set up used for rearing juvenile *Scylla serrata*

Initially feed was offered in split rations of 30 % at 0830 hrs and remaining 70 % at 1700 hrs, but based on feed consumption, feeding was restricted to a single ration and offered at 1700 hrs and the crabs were allowed to feed till the following day 0700 hrs, when the left-over feed, if any, was removed and 100% water exchange was done daily along with routine checking of water quality parameters.

The uneaten feed was collected in a filter cone of 100 µm mesh bolting silk set in a white plastic funnel (cone mouth 130 mm dia. and cone end 20 mm dia.), while siphoning out the water using a 15 mm dia. flexible PVC tubing. The wet weight of the left-over feed was noted and washed with double distilled water to remove any adhering salts and dried in an electric oven at 55 °C to achieve constant weight. The experiment was continued till the next moulting and further held for a period to get the shell hardened.

At the end of the feeding trial the crabs were individually tied to immobilise the claws using cotton thread, their live-weight taken and further euthanised (freeze killed), oven-dried and stored in a dessicator under refrigeration. Weende proximate principles, viz, dry matter, crude protein, crude fat, crude fibre, crude ash, acid insoluble ash, and nitrogen free extract (AOAC, 1990) of the dried feeds, left-over feed and crab samples were determined to study the response parameters (Figures 2-6).

3.3. Energy budgeting

3.3.1. Estimation of intake energy and growth

Thirty juveniles of *Scylla serrata* (av. body wt. 53.28 ±3.55 g, CW. 50.56 ±2.47 mm, Table-15) in the intermoult stage (Freeman *et al.*, 1987) were collected from the Chinese net operations from Manjanakkad, located on the east cost of Vypeen Island, near Cochin and transported to the laboratory as described in the previous experiment (3.2.). To ensure the uniformity in size, their carapace width and body weight were measured to the accuracy of 0.01 mm and 0.01 g. The crabs

were acclimatized to the laboratory conditions over a period of 10 days as mentioned in the previous experiment.

Feeding and water exchange were continued till the juvenile crabs moulted. Six of the newly moulted crabs were euthanated, oven dried at 55 °C and stored in a dessicator and kept under refrigeration, till biochemical analyses were conducted as mentioned in section 3.2.

Another six newly moulted crabs, ie., six replicates with one crab per replicate, were weighed and released in to 50 litre plastic troughs filled with 40 litres of fresh seawater of 28 ±1 ppt (Table-15). The feeding trials commenced on the day following moulting. The crabs were fed freshly shucked clam meat. The live clams were collected, depurated and shucked as mentioned in the earlier experiment.

Based on preliminary observations on the feed intake by the crabs a fixed ration of 7.8 % of body weight was offered to the crabs at 1700 hrs. The left-over feed was collected in the next morning by 0700 hrs along with 100 % water exchange. The wasted feed was collected into a filter cone of 100 µm mesh bolting silk set in white plastic funnel (cone mouth 130 mm dia. and cone end 20 mm dia.), while siphoning out the water using a 15 mm dia. flexible nylon tubing. Wet weight of the leftover feed was noted.

Defecation intensity was observed to be high immediately after the feed was offered at 1700 hrs and therefore fecal pellets were collected at 2 hourly intervals for the next 6 hours, to avoid the disintegration of fecal strands as a result of the increased activity of crabs in the night hours. The remaining fecal matter was collected in the morning prior to the removal of wasted feed and water exchange. Another peak intensity of defecation was observed immediately after 100 % water exchange was made at 0700 hrs and the faecal collection was continued for the next 2 hrs after water exchange. In the day time, during the observations, fecal matter found if any, was also collected and maximum care was taken to minimize the loss

of fecal samples. The fecal samples were siphoned using a 5 mm dia. flexible nylon air tubing, into a plastic funnel (cone mouth 80 mm dia. and cone end 15 mm dia.) fitted with a filter cone of 100 µm mesh bolting silk.

Freshly collected left-over feed as well as fecal samples were washed with double distilled water to remove any adhering salts, dried in an electric oven at 55 °C to achieve constant weight, and pooled in the samples from respective treatments for nutrient analysis.

After the second moulting, crabs and exuviae were weighed for the wet weight. The exuviae were oven-dried and subjected to proximate analysis. The newly moulted water crabs were released back in to the trough and further maintained for a period to harden the shell. The hardness of the new shell was checked by pressing the 2nd sternal plate with thumb by holding the crab in the right palm. Once the shell was fully hardened the weight and carapace width of the animals were noted. The crabs were then euthanated at -20 °C and oven-dried at 55 °C.

The desiccated samples of crab, exuviae, fresh feed sample, left-over feed and feces were pooled for each treatment and subjected to proximate analysis (3.6.1.) and the energy content was calculated.

3.3.2. Estimation of Metabolism and Excretion

3.3.2.1. Standard Metabolic Rate

The majority of the crabs were found to moult between 0700 hrs. and 1000 hrs., after the water exchange, particularly 1-2 days before and after the new-moon day. From the remaining 18 juvenile crabs of common animal pool, another 6 crabs (av. body wt. 53.28 ±3.55 g, CW. 50.56 ±2.47 mm), which moulted between 0700 hrs and 0900 hrs, in a span of 3 days, were selected for respirometry. The newly moulted crabs were allowed to starve for the next 20 to 22 hrs.

At the end of starvation the crabs were transferred to the plastic troughs with 30 l of fresh and aerated brackishwater of 28 ±1ppt. The water in each trough was sealed on top by pouring ≃ 30 ml of a 1:1 mixture of castor oil and sunflower oil (Dr. M.R. Anantharaman, Dept. of Physics, CUSAT, *Personal communication*) to prevent any exchange of gases between the water and the atmosphere. The experiment lasted for 24 hrs and 75 % of the water in the troughs was replaced with fresh, aerated, brackishwater of 28 ±1ppt salinity, at 3rd, 6th, 9th, 12th, 15th, 18th and 21st hrs causing little disturbance to the crabs. Temperature of the water was monitored at hourly intervals. Water samples were collected to estimate the initial and final dissolved oxygen (DO) and total ammonia-nitrogen (NH_3-N) levels prior to and after each water exchange, every 3 hourly intervals, using a 5 mm dia. flexible PVC tube to transfer the water sample into BOD bottles. Winkler's method (F.A.O., 1975) and phenol-hypochlorite method (Solorzano, 1969) were employed for the estimation of dissolved oxygen and total ammonia respectively. The differences in values were cumulatively processed to get the total basal dissolved oxygen intake (ml g^{-1} mid-body wt. hr^{-1}) and basal ammonia-nitrogen excretion (mg g^{-1} mid- body wt hr^{-1}) by the juvenile crabs.

Based on the feeding rate recorded over the moult cycle and physiological stages of moulting, it was assumed that the basal metabolic rate also changes and therefore the experiment was repeated twice, once at the middle of the inter-moult period and the other at the pre-moult stage using the same set of crabs.

3.3.2.2. Specific Dynamic Action

The experimental crabs used for the BMR trial were used for estimating the specific dynamic action. The feeding schedule and feed ration offered were same as described in 3.2.1.1. At 1700 hrs the feed was offered to the crabs and aeration was stopped. The water in each trough was sealed on top by pouring ≃ 30 ml of a 1:1 mixture of castor oil and sunflower oil to prevent any gas exchange with atmosphere. The water temperature in the troughs was monitored at hourly intervals. The procedure involved in the basal metabolic rate estimation was followed and the

Table-15: Details of experimental set up for energy budgeting studies in juvenile *Scylla serrata*

Total experimental duration: 69.67 ±1.63 days			
Biological parameters			
	Size at collection	Size at 1st moult	Size at 2nd moult
Average Body wt. (g)	53.28 ±4.55	84.27 ±2.09	146.48 ±1.83
Average CW (mm)	50.56 ±5.47	76.06 ±3.18	93.89 ±3.26
Moult stage	Throughout one moult cycle		
Water quality parameters			
Salinity (ppt)	28 ±1		
Temperature (°C)	23.6 ±3.5		
DO (ppm)	>4.0		
pH	8.0 ±0.5		
Total NH_3 (ppm)	< 0.05		
Statistical parameters			
6 replicates per treatment with each replicate having single crab			
Feed parameters			
Source	Freshly shucked meat of *V. cyprinoids*		
Feed ration	7.8% of body wt.		
Feeding schedule	Single ration at 1700 hrs, left to feed till it's removed next morning at 0700 hrs.		
Average feed offered per crab (g)	327.49 ±4.58		
Average feed intake per crab (g)	292.11 ±10.25		

dissolved oxygen uptake and ammonia-nitrogen excretion were recorded every 3 hours interval for a period of 24 hrs. Three blank troughs with similar quantity of feed as that of the feeding trials were set to estimate any feed associated changes in dissolved oxygen and ammonia-nitrogen in water. The dissolved oxygen and the total ammonia-nitrogen values obtained from the blank troughs were used to correct any feed associated effect in the troughs with crabs. The experiment was repeated thrice, viz., at the post-moult, mid-inter-moult and the pre-moult stages to assess the variation in the pattern of apparent specific dynamic action (ASDA) over the moult cycle.

In the energy budget estimations the following parameters were considered.

Assimilation Rate

AR = Food energy assimilated (Ae)/ (Live mid body wt × Days)

Ae = C - (F+U)

Energy Deposition on Growth

P = C- R + M + (F+U)

C- Food energy consumed

R – Metabolism

M – Moult loss

F – Faecal loss

U – Urinary loss

Assimilation Efficiency

AE = (Food energy assimilated ÷ Food energy consumed) × 100

Conversion Rate

CR = (Food energy converted or (P+M) ÷ Live mid body wt.)× 100

Gross Conversion Efficiency

GCE = (Food energy converted ÷ Food energy consumed) × 100

Net Conversion Efficiency

NCE = (Food energy converted ÷ Food energy assimilated) × 100

Feed Conversion Ratio

FCR = Wet body weight increment ÷ Dry feed weight

Energy is expressed in joules (J) and the rate of energy changes expressed as J g^{-1} mid-body weight hr^{-1} or day^{-1}.

Energy equivalent of 1 mg NH_3 is 20.5 J (Brafield, 1995) whereas 1 ml O_2 is equivalent to 20.098 J (Engelman, 1966).

3.4. Feeding trials with formulated pellet feed

3.4.1. Procurement, preparation and analysis of ingredients

3.4.1.1. Fish, squid, clam and shrimp meals

Fresh anchovy fish (*Stolephorus* sp.), purchased from the Ernakulam market was washed in potable water to remove any adhering contaminants such as sand particles. Fresh squid (*Loligo* sp.) purchased from the Murukkumpadom Harbour, Vypeen Island was beheaded, eviscerated and washed to remove any adhering dirt and ink material. Black clams (*Villorita cyprinoides*) were collected from the brackishwater ponds at Narakkal, Vypeen Island. The clams were allowed to depurate for 12 hrs, facilitating the faeces and mud egestion. The depurated clams were steam cooked for 20 minutes allowing the shell to open and the meat alone was collected.

All the three items were spread thinly on enameled metal trays, separately, and dried at 55 ±2 °C to reduce the moisture content to less than 8% and the dried materials were pulverized and sieved (250 μ sieve) to remove scales and bony materials. Sieved ingredients were stored in air-tight polyethylene bottles in a dessicator at room temperature.

For the preparation of shrimp meal, dry shrimp (*Penaeus indicus*) was procured from the Ernakulam fish market, removed the contaminants, if any, by visual observation, and dried in a hot air oven at 55±2 °C to reduce the moisture content ≤ 8 %. It was then pulverised and stored as mentioned above (Table-16).

3.4.1.2. Other ingredients

De-oiled and roasted soybean flour (Shakti Soy Foods Ltd.), whole-wheat flour, cod-liver oil (Seven Seas Ltd.), sunflower oil (Agro Tech Foods Ltd.) and soybean oil (Shakti Soya Foods Ltd.) were purchased from the local market.

Vitamin and mineral mixes (Catacutan, 2002; Cuzon and Guillaume, 1997) were formulated using the feed grade chemicals obtained from Merck India Ltd (Table-to 18 and 19). Guar gum, dextrin, cellulose, soy lecithin, and cholesterol were procured from Himedia Ltd.

The proximate analysis (Table-16) of ingredients, viz., fish meal, shrimp meal, clam meal, squid meal, roasted and de-oiled soybean flour, wheat flour etc. was done to estimate the nutrient and energy contents as presented in 3.6.1. (AOAC, 1990).

3.4.1.3. Formulation and preparation of diets

The formulation of the diet was done using Microsoft XP Excel software, taking the proximate composition and energy values and further optimizing them to the desired levels.

A common ingredient mix (CIM) was formulated using the protein rich ingredients - fish meal, squid meal, clam meal, shrimp meal and soybean flour (Table-17).

Using the proximate composition and energy data derived from CIM and other ingredients, specific feeds were designed and processed (Fig-1) and used in different feeding trials.

Table-16: Proximate composition of major ingredients used for the preparation of formulated diets (%, DM basis)

Ingredients	Crude protein	Crude lipid	Crude fiber	Crude ash	Acid insoluble ash	Nitrogen free extract
Fish meal	69.212	5.30	0.10	21.88	0.50	3.503
Squid meal	84.36	5.47	0.106	5.03	0.24	5.04
Shrimp meal	70.09	3.11	5.47	16.84	0.16	4.49
Clam meal	50.98	5.23	0	5.25	0.839	38.54
Soya flour (defatted)	52.87	1.34	1.59	9.54	0.04	34.67
Whole wheat flour	12.703	1.77	0.49	1.42	0	83.61
Dextrin	0.00	0.00	0.00	0.00	0.00	100.00
Cod liver oil / oil supplements	0.00	100.00	0.00	0.00	0.00	0.00
Lecithin	0.00	100.00	0.00	0.00	0.00	0.00
Cholesterol	0.00	100.00	0.00	0.00	0.00	0.00
Vitamin Mix	0.00	0.00	20.84	0.00	0.00	0.00
Mineral Mix	0.00	0.00	0.00	100.00	0.00	0.00
Guar gum	0.00	0.00	0.00	0.00	0.00	100.00

Table-17 Composition of common ingredient mix (CIM) used for the preparation of formulated diets (% DM basis)

Ingredient composition (% inclusion)					
Fish meal	Squid meal	Shrimp meal	Clam meal	Soya flour (defatted)	Total
55	3	7	20	15	**100**
Proximate composition (% DM basis, GE= 17.81 ±0.92 kJ/g)					
Crude protein	**Crude lipid**	**Crude fibre**	**Crude ash**	**Nitrogen free extract**	
63.01 ±1.03	4.54 ±0.18	0.624 ±0.00	16.85 ±1.35	14.97 ±1.17	

Table-18: Composition of vitamin mix used in formulated feed for juvenile *Scylla serrata*

Ingredients	mg/g
P-amino benzoic acid	6.67
D-Biotin	0.27
Inositol	266.67
Nicotinic acid	26.67
Calcium pantothenate	40.00
Pyrodoxine hydrochloride	8.00
Riboflavin	5.33
Cyanocobalamine	0.05
Folic acid	0.53
Choline chloride	400.00
Thiamine hydrochloride	2.67
Ascorbic acid	13.33
β Carotene	6.00
Retinone	1.00
Cholecalciferol	0.80
α-Tocopherol	13.33
Menadione	2.67
Cellulose	206.01

Table-19:Composition of mineral mix used in the formulated feed for juvenile *Scylla serrata*

Ingredients	mg/ g
K2HPO4	177.65
Na2HPO4. 7H2O	6.98
MgSO4. 7H2O	270.27
Ca3(PO4)2	241.42
CaCO3	303.67

Table- 20 Ingredient profile of formulated diets prepared (1) with graded protein levels and (2) with lipid supplements (as % inclusion)

Ingredients	Formulated diets having graded protein levels	Formulated diets having various lipid supplements
CIM	19.18 - 75.30	59.45
Wheat flour	10.00 - 24.17	24.00
Dextrin	0.80 - 45.48	0.00
Cod liver oil / oil supplements	2.91 - 5.44	6.00
Lecithin	1	1.00
Cholesterol	0.5	0.05
Vitamin mix	1.5	1.50
Mineral mix	9	5.00
Cellulose	0.89 - 1.4	1.00
Guar gum	2.00	2.00

Fig-1: Preparation of experimental diets

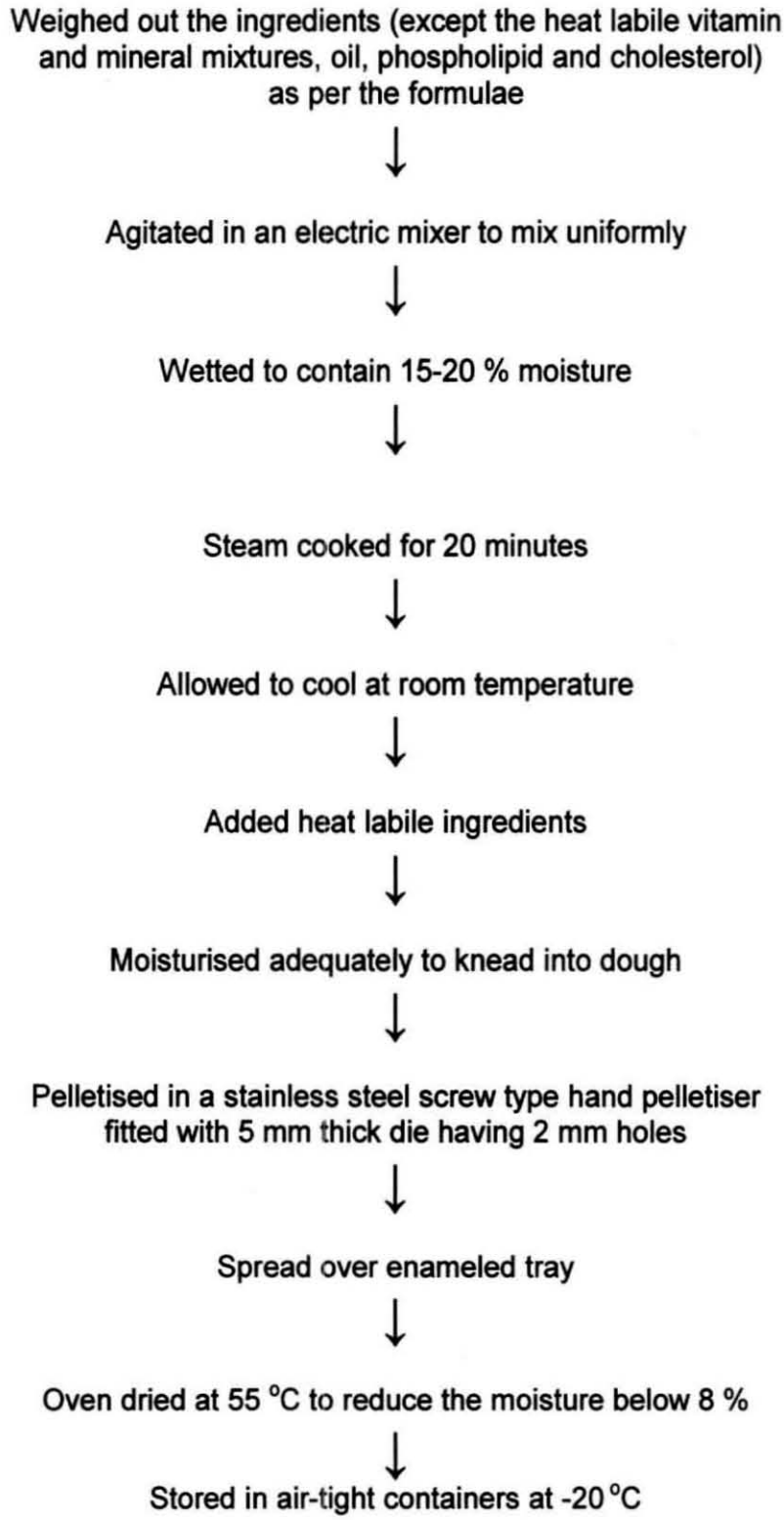


Table-21: Proximate composition of formulated iso-caloric and iso-lipidic diets with graded protein levels for juvenile *Scylla serrata* (% DM basis)

Diets	Crude protein	Crude lipid	Crude fibre	Crude ash	Nitrogen free extract	Gross energy (kJ/g)	Protein/ Energy (mg/kJ)
CP-15	49.18 ±0.86	8.00 ±0.09	1.86 ±0.01	18.28 ±1.21	22.67 ±1.59	17.44 ±0.36	28.20 ±0.08
CP-20	45.49 ±0.38	7.94 ±0.18	1.73 ±0.12	17.35 ±0.93	27.49 ±1.34	17.46 ±0.41	26.06 ±0.13
CP-25	40.61 ±0.70	7.98 ±0.39	2.23 ±0.08	15.87±1.19	33.31 ±2.31	17.44 ±0.29	23.30 ±0.09
CP-30	34.10 ±0.10	8.02 ±0.22	1.91 ±0.15	14.51 ±1.04	41.45 ±1.81	17.46 ±0.63	19.53 ±0.10
CP-35	30.35 ±0.53	8.06 ±0.86	1.88 ±0.06	13.58 ±1.09	46.13±2.06	1748 ±0.68	17.36 ±0.21
CP-40	24.78 ±0.77	8.10 ±0.13	0.62 ±0.03	16.14 ±0.85	50.35±2.51	17.03 ±0.25	14.55 ±0.11
CP-45	20.10 ±0.71	8.05 ±0.31	0.57 ±0.01	14.98± 1.00	56.29±1.86	17.03 ±0.84	11.80 ±0.38
CP-50	14.22 ±0.82	8.10 ±0.35	0.52 ±0.14	13.47 ±0.71	63.69±2.33	17.07 ±0.96	8.33 ±0.33

- CP-15, CP-20, CP-25, CP-30, CP-35, CP-40, CP-45 and CP-50 are formulated feeds containing 15-50 % crude protein in graded levels.

Table- 22: Composition of various lipid supplements used for the evaluation of different lipid sources for juvenile *Scylla serrata* (ml per 100 ml lipid supplement)

Oil sources	T1	T2	T3	T4	T5	T6
Cod liver oil	100.00	0.00	0.00	50.00	50.00	50.00
Sunflower oil	0.00	100.00	0.00	25.00	40.00	10.00
Soybean oil	0.00	0.00	100.00	25.00	10.00	40.00
Total	100.00	100.00	100.00	100.00	100.00	100.00
BHT (%)	0.01	0.01	0.01	0.01	0.01	0.01

BHT- Butylated hydroxyl toluene
T1 - Cod liver oil at 100%
T2 - Sunflower oil at 100 %
T3 - Soybean oil at 100 %
T4 - Cod liver oil : sunflower oil : soybean oil at 50:25:25 %
T5 - Cod liver oil : sunflower oil : soybean oil at 50:40:10 %
T6 - Cod liver oil : sunflower oil : soybean oil at 50:10:40 %

Table-23: Proximate composition of formulated diets supplemented with various lipid supplements (% DM basis)

Crude protein	Crude lipid	Crude fibre	Crude ash	Nitrogen free extract	Gross energy (kJ/g)	Protein/ Energy (mg/kJ)
40.61	**10.61**	**1.64**	**17.42**	30.47	17.66	23.00

3.4.2. Experiment to determine dietary protein requirement of juvenile crabs

Eight different feeds having dietary crude protein level ranging from 15% to 50% with 5% intervals (Table-20) were prepared by mixing the common ingredient mix (Table-17) with other ingredients

The details of experimental protocol are highlighted in Table-24. Mud crab juveniles, initial weight 0.25±0.051 g, collected from Narakkal brackishwater creek, were acclimated to the laboratory conditions for a period of 1 week. The crabs were reared individually in 35 litres of brackishwater kept in dark blue colored plastic troughs of 50 litre capacity. The crabs were weaned to a formulated pellet feed containing 45 % crude protein by starving them for a day and offering the pellet feed on the following day.

Each treatment had 6 replicates each with one crab. The feeding trial commenced from the day after moulting and continued for a period of 9 weeks. Feed was offered to the crabs as a single ration at 1700 hrs. The level was adjusted to 4.0 % based on preliminary observations on consumption.

Salinity, temperature, dissolved oxygen, total ammonia and pH were determined daily and were found to be 28 ±1 ppt, 27 ±4 °C, ≥5.5 ppm, ≤0.05 ppm and 8.0 ±0.5 respectively during the experimental period. About 75 % of the water was exchanged every day at 0700 hrs and the left-over feed accumulated in the bottom was siphoned out into the filter cone made of bolting cloth as mentioned in the earlier experiment (3.1.3.); further washed with double distilled water and oven-dried at 55 ±2 °C, pooled, separately for each treatment and the data on left-over feed were used to calculate the actual feed consumption on dry matter basis.

At the end of the feeding trial, the live weight and carapace width of the crabs were noted. The crabs were then subjected to euthanasia and oven-dried at 55±2°C. The proximate composition of feed samples and dried crab samples were determined (Fig-2-6). Amino acid profiles of the feeds (section 3.6.2.) were also determined to assess the protein quality of the feeds.

Table-24: Details of experimental set up for protein requirement study in juvenile *Scylla serrata* fed diets having graded protein levels

Total experimental duration: 63 ±3 days	
Biological parameters	
	Size at collection
Average Body wt. (g)	0.25 ±0.05
Average CW (mm)	10.0 ±0.02
Moult stage	Not determined
Water quality parameters	
Salinity (ppt)	28 ±1
Temperature (°C)	27.6 ±4.0
DO (ppm)	> 5.5
pH	8.0 ±0.5
Total NH_3 (ppm)	< 0.05
Statistical parameters	
Randomized block design	
6 replicates per treatment with one crab per replicate	
Feed parameters	
Source	Formulated feeds containing 15-50 % crude protein at 5 % graded levels. Crude lipid
Feed ration	Restricted 4% of body weight
Feeding schedule	Single ration at 1700 hrs, left to feed till it's removed next morning at 0700 hrs.

3.4.3. Evaluation of lipid supplements in formulated diets

A working volume of 50 ml each of cod liver oil, sunflower oil and soybean oil was transferred from the stock bottles in to 75 ml glass tubes with airtight lid. Butylated hydroxyl toluene (BHT, Himedia) was added at 0.02 % level to prevent oxidation. The 3 combinations of cod liver oil, sunflower oil and soybean oil were prepared along with 0.02 % of BHT, in another set of 3 tubes (Table-22). An iso-nitrogenous (40.61 % CP), iso-calorific (17.77 kJ g^{-1}) basal mix (4.61 % EE) was mixed with six oil supplements at 6 % level (Table-22) and processed into six diets and stored (Fig. 1). Six iso-nitrogenous (40.61% CP) and iso-caloric (17.66 KJ g^{-1}) diets, containing three lipid sources and their mixtures (viz., Diet T1-cod liver oil, Diet T2-sunflower oil, Diet T3-soybean oil, Diets T4, T5, and T6- mixture of cod liver oil with sunflower oil and soybean oil at 3 different ratios (Table-22) were prepared as described in the feed preparation section (3.4.1.).

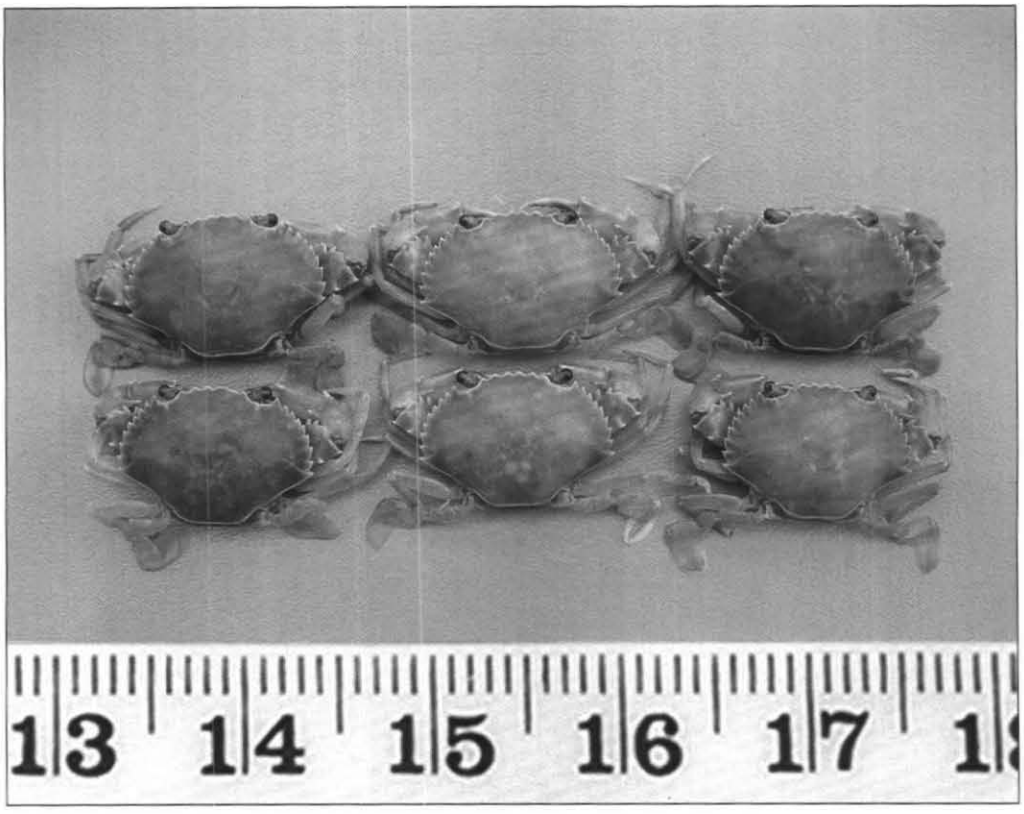
The details of experimental protocol are highlighted in Table 25. Juvenile crabs (average body wt. 0.28 ±0.03g, average carapace width 11.0 ±0.04 mm) were reared individually in 50 l capacity blue plastic troughs containing 30 l of water of salinity 28 ±1 ppt, temperature 25 ±4 °C, D.O. 5.3 ±0.3 ppm, total ammonia $\leq$0.05 ppm and pH 8.0 ±0.5 and weaned to the formulated diet by starving the crabs for one day and by offering the formulated feed on the following day as mentioned in the protein requirement experiment (section 3.4.2).

The feeds were offered at 4% of the body weight daily at 1700 hrs. The water exchange and waste feed collection protocols were as mentioned in protein requirement experiment (section 3.4.2.). The experiment was conducted for 9 weeks and at the end of the trial live weight and carapace width of all the crabs were processed for the biochemical analysis.

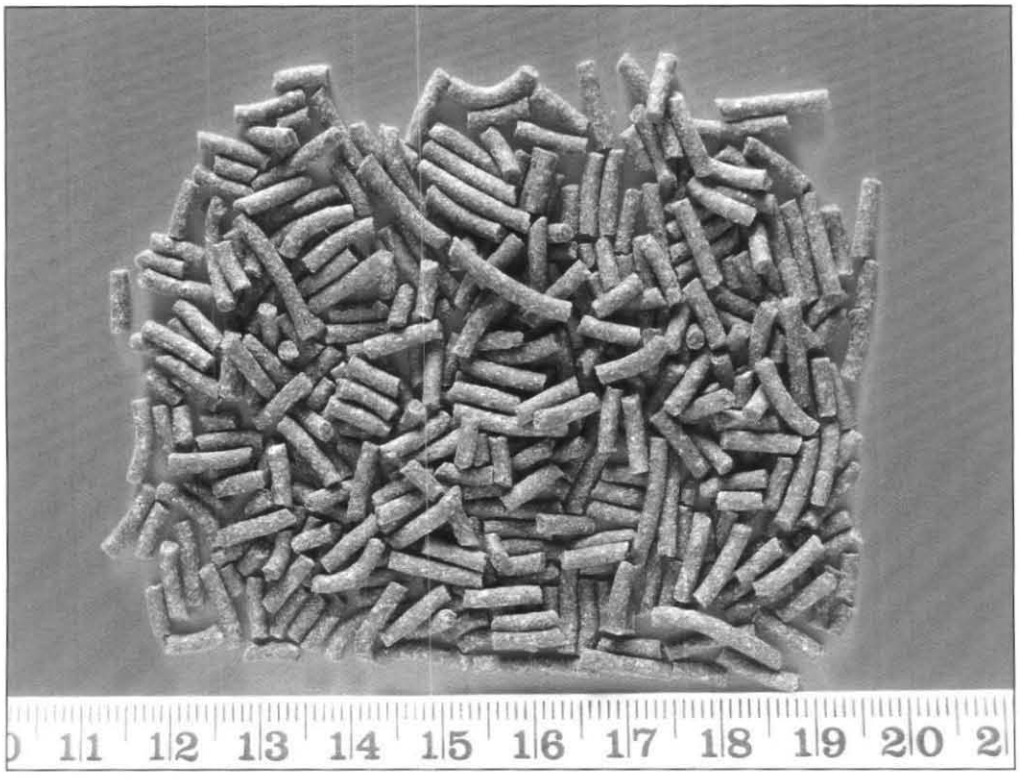
Table-25: Details of experimental set to evaluate various lipid supplements in the formulated diets fed to juvenile *Scylla serrata*

Total experimental duration: 63 ±3 days	
Biological parameters	
	Size at collection
Average Body wt. (g)	0.28 ±0.03
Average CW (mm)	11.0 ±0.04
Moult stage	Not determined
Water quality parameters	
Salinity (ppt)	28 ±1
Temperature (°C)	25.0 ±4.0
DO (ppm)	> 5.0
pH	8.0 ±0.5
Total NH_3 (ppm)	< 0.05
Statistical parameters	
Randomized block design	
6 replicates per treatment with one crab per replicate	
Feed parameters	
Source	Iso-nitrogenous (40.61%), iso-calorific (17.66 kJ/g) and iso-lipidic (10.61%) formulated feeds supplemented with cod liver oil, sunflower oil and soybean oil singly and oil mixtures containing above oils ratios of 2:1:1, 5:4:1 and 5:1:4 respectively.
Feed ration	Restricted at 4% of body weight
Feeding schedule	Single ration at 1700 hrs, left to feed till it's removed next morning at 0700 hrs.

PLATE-6



Scylla serrata **juveniles used for experimental trials with formulated diets**



Formulated pellet diet fed to juvenile *Scylla serrata* to study protein requirement and to evaluate various lipid supplements in diets

3.5. Response parameters

The following response parameters were estimated to assess the efficacy of various feeds.

Specific Growth Rate (SGR)

$$SGR = (\ln LW_1 - \ln LW_0) \times 100 / t$$

Percentage gain in live weight (LWG)

$$LWG = (LW_1 - LW_0) \times 100 / LW_0$$

Percentage gain in dry weight (DWG)

$$DWG = ((DW_1 - DW_0) \times 100 / DW_0$$

LW_0 - Initial live body weight

LW_1 - Final live body weight

DW_0 - Initial dry weight

DW_1 - Final dry weight

t - number of days

Percentage gain in carapace width (CWG)

$$CWG = (CW_1 - CW_0) \times 100 / CW_0$$

CW_0 – Initial carapace width

CW_1 – Final carapace width

Moulting Frequency (MF)

MF = Total Intermoult Duration / Number of ecdysis

Feed Conversion Ratio (FCR)

$$FCR = (LW_1 - LW_0) / \text{Feed intake (dry matter)}$$

Protein Efficiency Ratio (PER)

$$PER = (LW_1 - LW_0) / \text{Protein intake (dry matter)}$$

Survival Percentage

$$\% S = (\text{Final no. of stock} / \text{Initial no. of stock}) \times 100$$

3.6. Biochemical Analyses

3.6.1. Proximate composition

Proximate profiling of the dried (moisture <8%) feed samples were done following the AOAC method (AOAC, 1990). Flow charts for the proximate analyses are given in Fig. 2 to 6. The dry matter content of the feed and crab samples were determined by drying the samples in a hot-air oven at 100 ±4 °C for 2 hours (Fig.2). The crude protein estimation (Fig.3) was carried out by digesting the samples in a Kjeldhal digester followed by nitrogen distillation in a semi-automatic system (Kjelplus KPS-020, Pelican, Bio-innovations Pvt. Ltd.) and titrated against 0.1 N hydrochloric acid using an automated titration unit (Titroline-96, Schott). Crude lipid was estimated (Fig.4) by soxhlet extraction with petroleum ether (BP 40-60 ^{0}C) for 8 hours; the ash content (Fig.6) was determined as the residue remaining after incineration of samples at 550 ^{0}C for 1 hour in a muffle furnace. The lipid free sample after soxhlet extraction was further subjected to acid digestion followed by alkali digestion, drying and ashing to estimate crude fibre as weight difference before and after ashing. The nitrogen free extract (NFE) was computed by subtracting the percentages of crude protein, crude lipid, crude fibre and crude ash from 100 % dry matter.

3.6.2. Amino acid analysis

3.6.2.1. Sample preparation

About 0.1g feed sample with 10ml of 6N HCl was digested at 110° C in sealed tubes for 24 hours. The solution was filtered and flash evaporated thrice using distilled water to remove the acid. The acid-free sample was then made upto 5ml with 0.05N HCl, and filtered in a syringe nylon filter of 0.2µm. The pre-column derivatisation of amino acids was done with phenylisothiocyanite (PITC) to form phenylthiocarbamyl (PTC) amino acids, which can be detected with high sensitivity in a reversed-phase HPLC.

Fig-2: Proximate analysis - estimation of dry matter

Weighed a dry aluminum cup (W)

↓

Weighed of approx. 5g sample with cup (W_1)

↓

Kept in hot air at 60-70 °C till completely dry
(Modified method at CMFRI laboratories)

↓

Cooled in a dessicator and weighed to concordance (W_2)

↓

$$\% \text{ Dry matter} = \frac{W_2 - W}{W_1 - W} \times 100$$

Fig-3: Proximate analysis - estimation of crude protein

Weighed 0.5g of sample into the digestion tube

↓

Added 1g digestion mixture
(K_2SO_4+$CuSO_4$ in the ratio 9:1)

↓

Added 5 ml of concentrated H_2SO_4 and
digested in digester (Kjel-Plus) at 350°C

↓

Cooled to the tube room temperature after the solution
became clear and made up to 100 ml

↓

Took 10 ml of sample in the distillation tube and added 10 ml
40% NaOH in a Distiller (Kjel-Plus)

↓

Distilled for 6 minutes

↓

Collected the released ammonia vapour was in 10 ml boric acid solution
(4 % conc.) containing mixed indicator (Methyl red + Bromocresol green)

↓

The resultant green solution was titrated against 0.1N HCl
using an autotitrator (Titroline 96)

↓

$$\text{Crude Protein (\%)} = \frac{\text{Volume of acid (ml)} \times 0.0014 \times 100 \times 100 \times 6.25}{\text{Weight of sample (g)} \times 10}$$

Fig-4: Proximate analysis -estimation of crude fat

Weighed 3g sample into a thimble and plugged using absorbent cotton (W) and placed in a soxhlet extraction unit

↓

Weighed a round bottom flask (W_1)

↓

Assembled the flask in Soxhlet extraction unit

↓

Added petroleum ether (B.P. 40-60°C) to 1 1/2 times capacity of soxhlet

↓

Started water circulation in the condenser and set the hotplates on bench to 40-60°C

↓

Continued extraction for 8 hr (approx. 250 cycles)

↓

Removed the round bottom flask (RBF) from assembly and dried in hot air oven

↓

Cooled RBF in desiccator and weighed to concordance (W_2)

↓

$$\text{Crude Fat (\%)} = \frac{W_2 - W_1}{W} \times 100$$

Fig-5: Proximate analysis -estimation of crude fiber

Weighed 2 g fat free sample into 1 liter Berzelius spoutless beaker (W)

↓

Added 200 ml of 1.25% H_2SO_4, covered the mouth with RBF with cold water and digested for 30 minutes

↓

The digest was filtered through 100µm bolting silk by applying suction and repeatedly washed with warm water

↓

Residue was washed down with 200 ml 1.25% NaOH into the beaker for alkali digestion

↓

Filtered the contents into a Gooch crucible with a final rinse using acetone

↓

Oven dried the Gooch crucible at 60-70°C overnight (W_1)

↓

Ignited in a Muffle furnace at 550°C, Cooled to room temperature in a desiccator and weighed (W_2)

↓

$$\text{Crude Fibre (\%)} = \frac{W_1 - W_2}{W} \times 100$$

Fig-6: Proximate analysis -estimation of crude ash

Weighed clean silica crucible (W)

↓

Weighed approx. 3g sample with crucible (W_1)

↓

Kept in hot air oven at 60-70°C overnight and
Ignited in a muffle furnace at 550°C for 1 hour

↓

Cooled to room temperature in a desiccator and
weighed to concordance (W_2)

↓

$$\text{Crude Ash (\%)} = \frac{W_2 - W}{W_1 - W} \times 100$$

3.6.2.2. High Performance Liquid Chromatography (HPLC) analysis of amino acids

20 µl of sample was injected into HPLC (Waters reversed-phase PICO.TAG amino acid analysis system), fitted with packed column (PICO.TAG). The elution buffer used was sodium acetate trihydrate (pH 6.4) and acetonitrile. The detector (Waters 2487 dual ? absorbance detector) was set at 0.1 AUFS at 254nm and the column temperature was set at 38°C. Amino acid standard (PIERS Amino acid standard H) was run before each sample injection. Samples were injected in triplicate and the output was analyzed using Breeze software.

3.6.2.3. Estimation of Tryptophan

Tryptophan was estimated as per the spectrophotometric method (500nm) of Sastry and Tummura (1985) after alkali hydrolysis of the sample using 5% sodium hydroxide at 110° C for 24 hrs.

3.6.3. Fatty acid analysis

3.6.3.1. Sample preparation

Lipid in the feed samples was extracted by cold extraction method using chloroform-methanol mixture in 2:1 ratio (Folch *et al.*, 1957). Approx. 50 g feed sample was wetted to contain 80 % moisture, and made into a paste and 450 ml chloroform - methanol mixture (15 fold of the sample) with 0.01% BHT was added to the sample and homogenised thoroughly and filtered using Whatman No. 42 filter paper. The extraction was repeated three times with the same solvent mixture (total 15 folds of the wetted sample). The pooled filtrate was transferred to a clean separating funnel, into which double distilled water was added at 20 % of the filtrate volume. After mixing vigorously the contents were left undisturbed overnight under refrigeration for the complete separation of upper methanol, middle water and bottom chloroform layers. The polar residues diffused away in to the upper layers of water and methanol leaving the non-polar fatty acids in the bottom chloroform layer. The next morning the bottom chloroform layer was separated and filtered over anhydrous sodium sulphate to remove excess of moisture, transferred into a round

bottom flask and flash evaporated at 50 °C to remove excess of solvent. Further the contents were made up to 10 ml with chloroform.

3.6.3.2. Saponification and methylation

Five ml of 0.5% alcoholic potassium hydroxide solution was taken in a round bottom flask with approximately 2 ml of lipid sample. The contents were refluxed for 30 minutes and later 6 ml BF_3-Methanol (Sigma) was added and refluxed for 5 minutes. The whole process of refluxing was done in an atmosphere of nitrogen. The dry fatty acid methyl esters in the flask were extracted 2-3 times with petroleum ether quantitatively. The extract was further washed with 25 ml double distilled water three times and filtered over anhydrous sodium sulphate to remove any moisture. The solvent was then evaporated under a stream of nitrogen gas. The resultant product was transferred to smaller vials and stored at -20 °C until analyses.

3.6.3.3 Gas Chromatographic (GC) analysis of fatty acid methyl esters (FAME)

The fatty acid analysis was performed on a Perkin Elmer Auto-System XL, Gas Chromatograph (Perkin Elmer, USA), equipped with split / split-less injector. The detector used was a flame ionization detector (FID) and the column was Elite-5 (crossbond 5% diphenyl- 95% dimethyl polsiloxane) 30 meters long, 0.53 mm internal diameter and 0.50µm film thickness. The column temperature was programmed from 110°C to 160°C at 45°C/min and then to 250°C at 30°C/min and finally to 285°C at 15°C/min. The injector and detector temperatures were kept at 285°C and 290°C respectively. Nitrogen was used as the carrier gas with a pressure of 8psi. The flow rate of hydrogen and air were maintained at 50psi each. Standard fatty acid methyl ester mixture (Supelco, FAME mix C_4-C_{24}) was injected before running the samples. 0.5- 1µl of sample was injected in triplicate and the data acquisition was done with Total-Chrome 6.X.X software. Total run time per sample was set at 14 minutes

3.7. Statistical design of experiments and analysis of results

All the experiments had six replicates per treatment with one crab per replicate. Randomised block design was followed in the experiments to evaluate various diets (Snedecor and Cochran, 1967). Duncan's Multiple Range 'T' (DMRT) test was performed as the Post-Hoc test of ANOVA on the response parameters, to estimate the level of significance (95 % level). Multiple Linear Regression (MLR) analysis was performed on the proximate, amino acid and fatty acid profiles of the feeds against the response parameters in order to find out the feed associated predictors of growth response. Both DMRT and MLR were performed on SPSS-10 processor. The optimum level of crude protein requirement was calculated from the mean values of response parameters by polynomial regression method using Curve Fit-1.3 processor. All statistical analytical protocols, except polynomial regression were performed on the raw data of various parameters estimated.

CHAPTER IV
Results

4. RESULTS

4.1. Outcome of survey on mud crab culture and feed management

The results of the farm survey are highlighted in Table 26. The water spread area of farms surveyed ranged from 0.3 to 2 ha and the depth from 0.5 to ≤1 m. The majority of farms were observed to follow systematic preparation and maintenance of ponds, stocking of crabs and water quality management as done in shrimp farming. The salinity, temperature and pH in the farms selected for observations were in the range 13 to 30 ppt., 20 to 33 °C, 7.9 to 8.5 respectively (Plates 1 & 2, Table 26).

Among the crab farms surveyed (Table 26), some were exclusively practising fattening culture, where 'water crabs' (newly moult crab) ≥ 250 g and were stocked at a density of 0.5-1/ m^2; only few farms were observed to exclusively doing grow-out culture, where juvenile mud crabs of 50 to 200 g size were stocked at a rate of 2 to 5/m^2 numbers depending on the size. Another method was mixed type farming, i.e., both fattening and grow-out culture were done together or begin the season with grow-out culture followed by fattening culture as the juvenile crabs attained a size ≥ 250 g. Fattening of mud crabs was also done in the traditional prawn filtration systems. Another crop-rotation method was to begin the season with the fattening culture of mud crabs for 2 to 3 months (September-November) and after harvest during the remaining season (December-May) a crop of *Penaeus indicus* was taken in areas where the salinity reached 30-34 ppt.

The most common feeds fed to crabs were trash fish stored in brine; boiled clam meat and slaughterhouse waste, and rarely fresh trash fish and salt-dried trash fish. Indiscriminate feeding with spoiled and poor quality feeds available at lowest price was seen in most of the farms. Spoiled fish procured and stored repeatedly in the same brine solution was the most commonly used feed in the farms, which was less attractable to crab. As a result feed wastage and water quality spoilage were noted. Quality of feed and feeding schedules were seen compromised for the cost,

Table-26: Mud crab farming and feeding practices in selected farms in Ernakulam and Allepy districts in Kerala state

Farm	1	2	3	4	5	6	7	8
Location	Vypeen	Vypeen	Vypeen	Panamukad	Alleppy	Alleppy	Alleppy	Alleppy
W.S. Area	1.5 acres	0.5 acre	1.0 acre	2.0 acres	1.5 acres	0.33 acre	0.73 acre	1.0 acre
Type of culture	Fattening	Mixed	Fattening	Grow out	Fattening along with Tradition-al shrimp farming	Fattening along with second crop of *P. indicus*	Fattening	First 4 months grow out followed by fattening
Seed size	250 g and above	All sizes	250 g and above	50-200 g	250 g and above	250 g and above	250 g and above	50-200g for grow out and 250g and above for fattening
Stocking density	Not known (Continu-ous stocking and harvest-ing)	Continu-ous stock-ing and harvesting	Continu-ous stocking and harvesting	2-3/m^2	Not known	Not known	0.5/m2	3-4/m^2 for grow out & 0.5/m^2 for fattening
Feed & Feeding	Salted trash fish, Fresh Tilapia and occasion-ally clam meat	Salted trash fish Fresh Tilapia and occasion-ally clam meat	Salted trash fish	Salted trash fish	Salted trash fish and clam meat	Salted trash fish and clam meat	Salted trash fish, clam meat and occasion-ally chicken visera	Salted trash fish, clam meat and occasion-ally chicken visera
Water exchange	Tide fed	Tide fed	Tide fed	Tide fed	Tide fed	Tide fed	Tide fed	Tide fed

availability and convenience. Lack of awareness among the farmers on the importance of quality and proper storage of feeds, feeding strategies and feed management was noted.

Loss of stock due to the escapement and cannibalism was common as a result of untimely feeding schedules and insufficient feed rations. During the summer months (April-May) high mortality rates were seen in the crab farms and also harvested crabs during the transportation due to excessive heat, may be accounted towards the poor health conditions of the stock, which in turn may be a result of poor quality feeds and improper feed management.

4.2. Evaluation of fresh and processed feeds

4.2.1. Proximate profile of the fresh and processed feeds

4.2.1.1. Crude protein

The proximate analysis (Table-27) showed significantly ($P<0.05$) higher crude protein content (58.95-59.06 %) in the fresh, frozen and dried clam meat (*Villorita cyprinoides*) than in salted fish, SCF-3, SCF-2, frozen fish and SCF-1 (55.22 %). There were no significant ($P>0.05$), differences in crude protein content among the processed and fresh clam feeds, but the protein content of clam feeds was significantly ($P<0.05$) different from the other feeds. Crude protein content varied significantly ($P<0.05$) between frozen and salted fish, but not significantly ($P>0.05$) different from that of SCF-1, SCF-2 and SCF-3. Similarly protein content of salted fish and SCF-3 were not significantly different ($P>0.05$).

4.2.1.2. Crude lipid

The highest crude lipid content (Table-27) was found in frozen fish (26.01%) followed by salted fish and the combination diets, and the lowest lipid levels were found in clam feeds (9.01-10.01%). There were significant (*$P<0.05$*) differences in the lipid levels among most of the feeds, except among dry, frozen, and fresh clam meat.

4.2.1.3. Crude fiber

The crude fiber levels (Table-27) ranged from 0.01 % to 3.14 % in the feeds with the lowest level in clam feeds (0.01%) and the highest in SCF-1 (3.14%), followed by SCF-2, SCF-3, frozen fish and salted fish (1.40%) and the crude fiber levels varied significantly ($P<0.05$) among the feeds, except for clam feeds. The crude fiber content of clam feeds and combination diets varied significantly ($P<0.05$) from frozen fish, where as SCF-2 and SCF-3 did not vary significantly ($P>0.05$) from salted fish in their crude fiber contents.

4.2.1.4. Crude ash

The crude ash levels (Table-27) varied significantly ($P<0.05$) among the feeds except for clam feeds. The crude ash levels were found to be significantly ($P<0.05$) higher in the salted fish (20.25 %), owing to the absorption of common salt used for preservation by the fish tissues. Frozen fish (13.38%) had the next highest level of crude ash followed by combination diets, and the clam feeds (9.01-9.16%) had significantly ($P<0.05$) lower crude ash content.

4.2.1.5. Nitrogen free extract (NFE)

The highest NFE levels (Table-27) were found in the clam feeds (26.64-26.78%) followed by combination diets and frozen fish and the lowest ($P<0.05$) NFE levels were recorded in salted fish (0.61%). The NFE levels also showed significant ($P<0.05$) difference among the feeds except for the clam feeds.

4.2.1.6. Gross energy

The gross energy content (Table-27) of feeds ranged between 14.67 and 16.24 kJ/g and did not show any significant ($P>0.05$) influence on the growth performance of the crabs. Also the energy content of clam feeds (fresh, frozen and dried clam meat, $P>0.05$) and combination feeds (SCF-1, SCF-2 and SCF-3, $P>0.05$) did not vary significantly ($P>0.05$).

Table-27• Proximate composition and energy content of the fresh and processed natural feeds •(% dry matter basis)

Feeds	Crude protein	Crude lipid	Crude fiber	Crude ash	Nitrogen free extract	Gross energy (kJ/g)
Fresh clam	58.69 ±0.73^{a}	9.16 ±0.09^{a}	0.01 ±0.00^{a}	5.45 ±0.03^{a}	26.69 ±0.71^{a}	15.17 ±0.16^{a}
Frozen clam	59.06 ±0.92^{a}	9.01 ±0.40^{a}	0.01 ±0.00^{a}	5.29 ±0.11^{a}	26.64 ±0.71^{a}	15.18 ±0.33^{a}
Dry clam	58.93 ±0.23^{a}	9.03 ±0.22^{a}	0.01 ±0.00^{a}	5.25 ±0.01^{a}	26.78 ±0.01^{a}	15.18 ±0.28^{a}
Frozen fish	55.46 ±0.51^{bd}	26.00 ±0.26^{b}	1.93 ±0.12^{b}	13.38 ±0.20^{b}	3.23 ±0.22^{b}	16.24 ±0.06^{b}
Salted fish	57.38 ±0.50^{c}	21.95 ±0.27^{c}	1.40±0.14^{c}	18.66 ±0.29^{c}	0.61 ±0.05^{c}	14.67 ±0.31^{c}
SCF 1*	55.22 ±0.29^{b}	20.84 ±0.55^{d}	3.14 ±0.09^{d}	12.28 ±0.15^{d}	8.52 ±0.23^{d}	15.38 ±0.21^{a}
SCF 2*	56.27 ±0.56^{d}	16.43 ±0.46^{e}	2.82 ±0.07^{e}	11.18 ±0.82^{e}	13.29 ±0.25^{e}	15.01 ±0.22^{ac}
SCF 3*	56.42 ±0.49^{cd}	14.95 ±0.23^{f}	2.45 ±0.13^{f}	10.29 ±0.78^{f}	15.89 ±0.10^{f}	14.93 ±0.26^{ac}

- * SCF-1, SCF-2 and SCF-3 are combination diets containing shrimp head, clam meat and oil sardine, all frozen, at 1:2:7, 1:4:5 and 1:5:4 respectively

4.2.2. Amino acids profile

The ratio of total essential amino acid content to total non-essential amino acid levels (Table-28) ranged between 0.98 and 1.14. The frozen and salted fish and combination feeds had EAA/NEAA ratio above 1 (1.00-1.14) where as the ratio in clam feeds was less than 1 (0.98). The total essential amino acid levels and total non-essential amino acids levels (Table-28) of the feeds were 24.60-28.33 % and 22.38-28.93 % respectively, except for the salted fish, which recorded lower levels, viz. 21.05 % and 20.46 % respectively. Significant reduction in essential amino acids such as isoleucine, leucine, lysine, threonine and phenylalanine as well as non-essential amino acids such as cystine, glutamic acid, glycine and proline were observed in salted fish than frozen fish. The calculated non-protein nitrogen percentage was also found to be high (15.88 % of dry matter) in the salted fish.

4.2.2.1. Essential amino acids

Significant ($P<0.05$) differences were found among the amino acid profiles(Table-28) of the selected feeds. The lowest (2.42-2.43 %) and the highest (3.59 %) arginine contents were observed in clam diets and frozen fish respectively. Frozen fish had the highest histidine (3.09 %) content followed by SCF-1, salted fish, SCF-2 and SCF-3, where as, the lowest histidine content was found in clam feeds (2.44-2.47 %). Clam feeds (2.10-2.11 %) had the highest isoleucine content followed by the combination diets and frozen fish. Significant reduction in isoleucine was recorded in salted fish which was half (0.96 %) of that found in frozen fish. Similarly, the highest leucine content was seen in clam feeds (4.76-4.77 %) followed by combination diets and frozen fish and the lowest in salted fish (2.77 %). Except for salted fish (4.16 %) the lysine content in all the feeds ranged from 4.91 to 5.90 %. In frozen and salted fish the methionine level was in the range of 1.50-1.55 %, where as in all other feeds it ranged from 1.11 to 1.29 %, with clam feeds having the lowest level. Threonine level was lowest in salted fish (1.86 %) and highest in clam feeds (4.02-4.05 %). The lowest tryptophan levels were recorded in clam feeds (0.52-0.53 %), where as frozen fish (1.05 %) had the highest tryptophan level. Phenylalanine

Table-28: Amino acid composition of fresh and processed feeds fed to juvenile *Scylla serrata* (% DM)

Amino acid	Fresh clam	Frozen clam	Dry clam	Frozen fish	Salted fish	SCF-1*	SCF-2*	SCF-3*
Arginine	*2.42*	*2.42*	*2.43*	*3.59*	*2.82*	*2.95*	*2.74*	*2.62*
Histidine	*2.45*	*2.47*	*2.44*	*3.09*	*2.69*	*2.61*	*2.51*	*2.44*
Isoleucine	*2.11*	*2.10*	*2.11*	*1.82*	*0.96*	*1.66*	*1.72*	*1.74*
Leucine	*4.76*	*4.77*	*4.77*	*3.31*	*2.77*	*3.20*	*3.50*	*3.63*
Lysine	*5.24*	*5.26*	*5.26*	*5.90*	*4.16*	*5.10*	*5.00*	*4.91*
Methionine	*1.11*	*1.13*	*1.11*	*1.55*	*1.50*	*1.29*	*1.22*	*1.17*
Threonine	*4.02*	*4.05*	*4.02*	*2.58*	*1.86*	*2.56*	*2.85*	*2.98*
Tryptophan	*0.52*	*0.53*	*0.52*	*1.05*	*0.93*	*0.83*	*0.73*	*0.68*
Phenylalanine	*2.88*	*2.88*	*2.89*	*2.18*	*1.71*	*2.06*	*2.20*	*2.26*
Valine	*2.72*	*2.73*	*2.73*	*2.62*	*1.64*	*2.34*	*2.37*	*2.37*
Alanine	4.85	4.88	4.87	2.71	2.66	2.80	3.23	3.43
Aspartic acid	4.81	4.85	4.83	5.57	5.47	4.79	4.68	4.59
Cystine	0.21	0.21	0.21	0.90	0.56	0.67	0.54	0.47
Glutamic acid	4.37	4.36	4.37	7.95	5.49	6.36	5.70	5.32
Glycine	8.28	8.39	8.36	2.09	1.74	3.03	4.26	4.86
Proline	0.70	0.67	0.68	1.07	0.64	0.87	0.80	0.75
Serine	2.85	2.86	2.88	2.17	2.16	2.05	2.19	2.25
Tyrosine	2.71	2.70	2.72	1.89	1.73	1.82	1.99	2.06
S EAA	28.22	28.33	28.30	27.70	21.05	24.60	24.85	24.80
S NEAA	28.78	28.93	28.92	24.35	20.46	22.38	23.38	23.73
EAA/NEAA	0.98	0.98	0.98	1.14	1.03	1.10	1.06	1.05
Non protein nitrogen (calculated)	1.70	1.81	1.71	3.41	15.88	8.23	8.04	7.89

- * SCF-1, SCF-2 and SCF-3 are combination diets containing shrimp head, clam meat and oil sardine, all frozen, at 1:2:7, 1:4:5 and 1:5:4 respectively
- Values given in italics are essential amino acids.

content ranged from the lowest in salted fish (1.71 %), to the highest in clam feeds (2.88-2.89 %) followed by the combination diets (2.06-2.26 %) and frozen fish (2.18 %).

4.2.2.2. Non-essential amino acids

Among the non-essential amino acids (Table-28), clam feeds had the highest glycine (8.28-8.39 %), alanine (4.85-4.87 %), serine (2.85-2.86 %) and tyrosine (2.70-2.72 %) levels; frozen fish had the highest glutamic acid (7.95 %). aspartic acid (5.57 %), and cystine (0.90 %), where as the combination diets had higher proline levels (1.28-1.32 %).

4.2.3. Fatty acids profile

The highest content of saturated fatty acids (Table-29) was observed in salted fish (13.56%), followed by frozen fish; the saturated FA levels were (4.35-4.88 %) low in combination diets and the clam feeds .The total content of unsaturated fatty acids was the highest in the frozen fish and the lowest was found in clam feeds (4.15-4.81 %). Higher percentage of mono-unsaturates was recorded in frozen fish (7.31 %) followed by salted fish (6.32 %) and SCF-1 (6.09 %), moderate amounts in SCF-2 and SCF-3 (4.20-4.74 %) and the lowest levels in clam feeds (2.44-2.69 %). In the case of PUFA's, the frozen fish had higher PUFA level (2.68 %) followed by the combination diets (1.701-2.20 %) and salted fish (1.34 %), with the lowest PUFA level in clam feeds (0.91-0.94 %). n3 fatty acid levels (Table-29) ranged between 1.27 % and 6.44 % with n3 being more in frozen fish followed by SCF-1> SCF-3> SCF-2> fresh clam> frozen clam> dry clam and lowest n3 in salted fish (1.34 %), and n6 levels ranged from 0.79 to 1.27 % being more in combination diets (1.09-1.27 %) followed by frozen fish (0.99 %) and clam feeds (0.79-0.83 %). The lowest n6 level was found in salted fish (0.69 %). The highest n3 HUFA content (Table-29) was observed in frozen fish (4.62 %) and the lowest was found in dry clam meat (0.61 %) and salted fish (0.71 %). The ratio of saturated to unsaturated fatty acids

was relatively low in frozen fish and combination diets (0.77-0.82), where as salted fish had the ratio (1.61). The n3/n6 ratio was found to be higher in frozen fish (6.51 %) followed by combination diets (2.90-3.72), while the lowest ratio was recorded in dry clam (1.60).

4.2.3.1. Essential fatty acids

Linoleic acid (LA, 18:2n6): 18:2n6 levels (Table-29) fluctuated between 0.22-0.61 % of the total fatty acids in the feeds with the highest level in frozen fish (0.61%) followed by salted fish (0.46 %), and the combination diets (0.38-0.44 %) and the lowest 18:2n6 level was in clam feeds (0.22 %).

Linolenic acid (LNA, 18:3n3): Frozen fish had the highest content (1.73 %) followed by combination diets (1.02-1.33 %) and relatively lower levels were recorded in salted fish (0.69 %) and clam feeds (0.63-0.64 %).

Arachidonic acid (AA, 20:4n6): Diet SCF-1 had the highest arachidonic acid content (0.39 %) followed by frozen fish (0.31 %) and very low levels were recorded in the clam feeds (0.03-0.06%).

Eicosapentaenoic acid (EPA, 20:5n3): The highest EPA content was found in frozen fish (2.16 %), followed by the combination feeds (0.78-1.26 %), and clam feeds and salted fish had low EPA content (0.15-0.54 %).

Docosahexaenoic acid (DHA, 22:6n3): As in the case of EPA, DHA was also high in frozen fish (2.46 %) followed by the combination diets (1.49-2.07 %) and the lowest DHA level was recorded with salted fish (0.18 %).

DHA/ EPA ratio: DHA/EPA ratio (Table-29) in the combination diets ranged from 1.64 to 1.91 which are very close to the ideal ratio of 2 recommended for crustacean species. The lowest ratio was found with salted fish (0.33) where as clam feeds (3.07-4.70) had substantially high ratios.

Table-29: Fatty acids profile of fresh and processed feeds used for juvenile *Scylla serrata* (% of DM)

Fatty acid	Fresh clam	Frozen clam	Dry clam	Frozen fish	Salted fish	SCF-1*	SCF-2*	SCF-3*
C12:0	0.01	0.02	0.03	0.08	0.07	0.07	0.05	0.05
C14:0	0.56	0.55	0.61	1.72	1.97	1.28	1.00	0.90
C14:1n7	0.03	0.03	0.04	0.07	0.06	0.05	0.05	0.03
C15:0	0.09	0.10	0.08	0.17	0.16	0.14	0.12	0.12
C16:0	2.18	2.29	2.54	6.06	6.91	4.82	3.78	3.42
C16:1n7	1.21	1.05	1.18	2.73	2.81	2.22	1.86	1.69
C16:2n6	0.04	0.04	0.03	0.03	0.02	0.02	0.04	0.04
C17:0	0.59	0.67	0.66	0.34	1.56	0.53	0.59	0.62
C17:1n6	0.53	0.52	0.48	0.04	0.03	0.41	0.40	0.46
C18:0	0.92	0.96	0.95	2.91	2.88	2.32	1.80	1.61
C18:1n9	0.39	0.36	0.34	3.91	3.02	2.78	1.81	1.41
C18:1n7	0.22	0.19	0.25	0.02	0.03	0.17	0.21	0.22
C18:2n6	*0.22*	*0.22*	*0.22*	*0.61*	*0.46*	*0.44*	*0.38*	*0.42*
C18:3n3	*0.64*	*0.63*	*0.63*	*1.73*	*0.69*	*1.33*	*1.06*	*1.02*
C18:4n3	0.03	0.02	0.03	0.02	0.03	0.04	0.04	0.03
C20:1n11	0.26	0.25	0.26	0.44	0.33	0.41	0.36	0.35
C20:4n6	*0.05*	*0.03*	*0.06*	*0.31*	*0.17*	*0.39*	*0.27*	*0.24*
C20:5n3	*0.23*	*0.18*	*0.15*	*2.16*	*0.54*	*1.26*	*0.91*	*0.78*
C22:1n11	0.03	0.03	0.03	0.04	0.03	0.03	0.03	0.02
C22:6n3	*0.93*	*0.85*	*0.46*	*2.46*	*0.18*	*2.07*	*1.65*	*1.49*
C24:1n3	0.02	0.02	0.00	0.06	0.00	0.01	0.01	0.02
Σ Saturated	4.35	4.59	4.88	11.27	13.56	9.17	7.33	6.72
Σ unsaturated	4.81	4.42	4.15	14.64	8.40	11.64	9.09	8.21
Σ MUFA	2.69	2.44	2.57	7.31	6.32	6.09	4.74	4.20
Σ PUFA	0.93	0.91	0.94	2.68	1.34	2.20	1.75	1.70
Σ n3	1.84	1.70	1.27	6.44	1.43	4.71	3.67	3.34
Σ n6	0.83	0.81	0.79	0.99	0.69	1.27	1.09	1.15
Σ n3 HUFA	1.16	1.03	0.61	4.62	0.71	3.32	2.56	2.27
Saturated : Unsaturated	0.90	1.04	1.18	0.77	1.61	0.79	0.81	0.82
n3 : n6	2.21	2.10	1.60	6.51	2.08	3.72	3.37	2.90
DHA : EPA	4.03	4.70	3.07	1.14	0.33	1.64	1.80	1.91

- * SCF-1, SCF-2 and SCF-3 are combination diets containing shrimp head, clam meat and oil sardine, all frozen, at 1:2:7, 1:4:5 and 1:5:4 respectively.
- Values given in italics are essential fatty acids.

4.2.4. Survival

100 % survival was recorded in all the treatments through out the experiment.

4.2.5. Growth response

4.2.5.1. Weight gain

The combination feeds provided the best weight gain (wet wt. gain=81.52-85.20%, dry wt. gain=99.18-103.07%) in crabs followed by fresh and frozen clam (wet wt. gain=69.90-71.39%, dry wt. gain=54.98-57.50%), frozen fish (wet wt. gain=53.74%, dry wt. gain=44.25%), dry clam (wet wt. gain=49.83%, dry wt. gain=15.66%) and the lowest weight gain was noted in crabs fed the salted fish (Table-30, Fig. 7).

In the case of wet weight gain, no significant ($P>0.05$) difference was observed between fresh and frozen clam, between dry clam and frozen fish and among the combination diets, where as wet weight gain significantly ($P<0.05$) varied between fresh and frozen clam, dry clam and frozen fish, salted fish and combination feeds. The dry weight gains among, fresh and frozen clam, and among combination diets were not significantly ($P>0.05$) different.

4.2.5.2. Carapace width gain

The crabs reared on combination diets (SCF-1, SCF-2 and SCF-3) had wider carapaces (Table-30, Fig.7) and there were no significant ($P>0.05$) difference among the three combination diets. This was followed by fresh clam and frozen clam (14.88-16.10%, $P>0.05$), dry clam and frozen fish (11.87-12.17%, $P>0.05$), and the lowest carapace width gain was observed in crabs fed salted fish (8.73%, ($P<0.05$).

4.2.5.3. Specific growth rate

Significantly ($P<0.05$) higher SGR (1.00-1.03%) was recorded in crabs fed the combination diets (Table-30, Fig.8) followed by fresh and frozen clam feeds (0.69-0.72%), frozen fish and dry clam feed (0.36-0.41%) and the lowest SGR with salted

fish (0.23%). The SGR values were not significantly (P>0.05) different among fresh and frozen clam, dry clam and frozen fish, and combination diets, but varied significantly (P<0.05) among fresh and frozen clam, dry clam and frozen fish, and among salted fish and combination feeds.

4.2.5.4. Feed conversion ratio

The lowest FCR (Table-30, Fig. 8) indicating the best feed efficiency was recorded with combination diets (1.17-1.27, P>0.05) followed by fresh and frozen clam (1.76-1.78, P>0.05), frozen fish (3.04, P<0.05), dry clam (5.18, P<0.05) and the highest FCR suggesting poor feed conversion efficiency was with salted fish (5.44, P<0.05).

4.2.5.5. Protein efficiency ratio

The best PER (Table-30, Fig. 8) was obtained with the combination diets (1.45-1.52, P>0.05), followed by fresh and frozen clam (0.95-0.96, P>0.05), frozen fish (0.59, P<0.05) and relatively poor PER was recorded with dry clam and salted fish (0.32-0.33, P>0.05).

4.2.5.6. Intermoult duration

Intermoult duration was relatively shorter (Table-30, Fig. 9) for the crabs fed combination diets SCF-2 and SCF-3 (52.00-54.00 days, P>0.05), followed by fresh and frozen clam (63.00-64.00 days, P>0.05), frozen fish (91.00, P<0.05), dry clam (95.00, P<0.05) and the longest intermoult period was recorded with salted fish (121.00 days) which was significantly higher than all other feeds (P>0.05), except for dry clam (P<0.05).

4.2.6. The proximate profile of the experimental crabs

4.2.6.1. Moisture & Dry matter

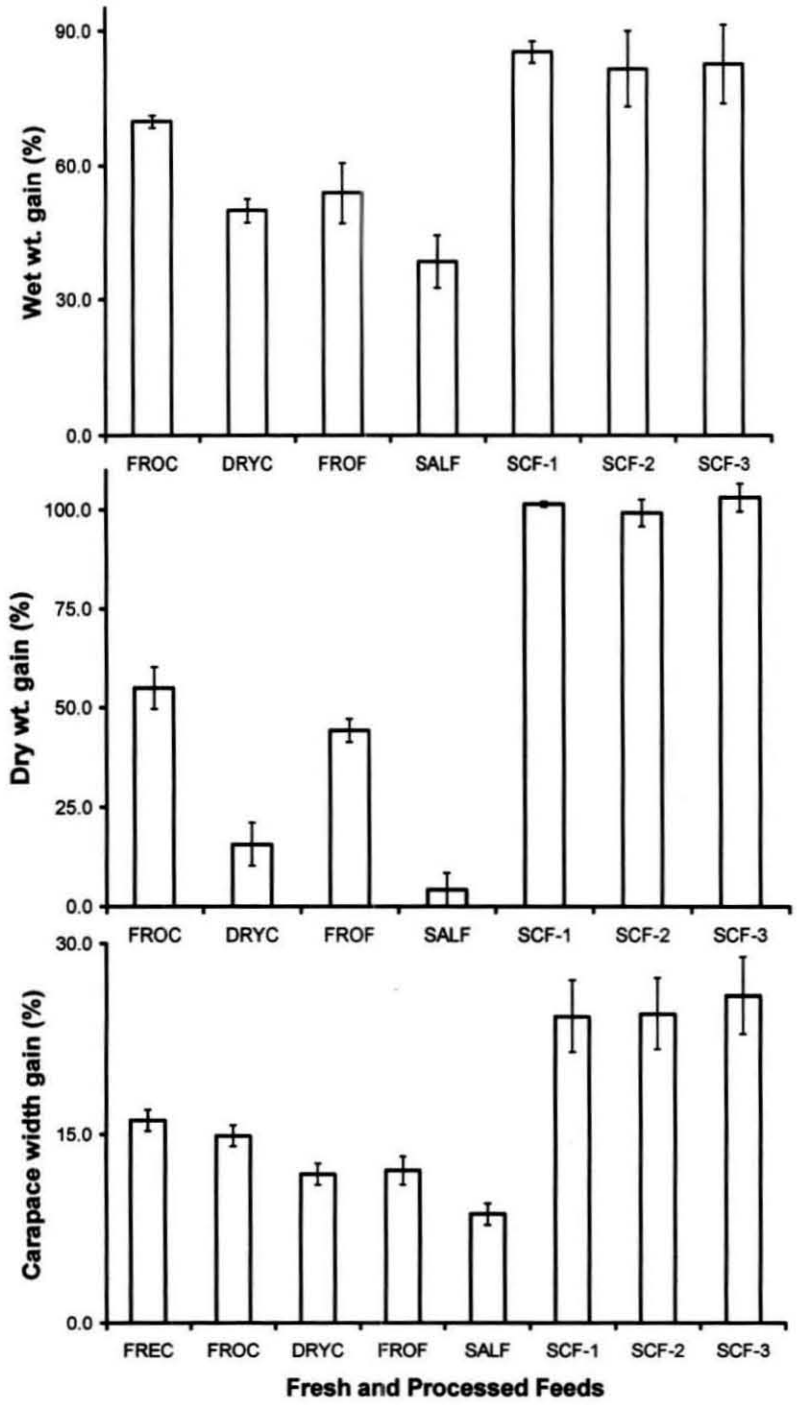
The lowest moisture and highest dry matter levels (Table-31, Fig.10) were noted in crabs fed the combination diets (M=69.71-70.39%, DM=29.61-30.29%, P>0.05), followed by frozen fish (M=74.45%, DM=25.55%, P<0.05) fresh and frozen

Table-30: Growth response of juvenile *Scylla* serrata fed fresh and processed feeds

FEEDS	% SGR	FCR	PER	% WET WT. GAIN	% DRY WT. GAIN	% CW GAIN	INTERMOULT DURATION (days)
Fresh Clam	0.72 ±0.02 [a]	1.76 ±0.05 [a]	0.96 ±0.16 [a]	71.39 ±1.36 [a]	57.50 ±5.27 [a]	16.10 ±0.85 [a]	64.00 ±0.63 [a]
Frozen Clam	0.69 ±0.04 [a]	1.78 ±0.06 [a]	0.95 ±0.10 [a]	69.90 ±2.66 [a]	54.98 ±5.43 [a]	14.88 ±0.82 [a]	63.00 ±1.41 [a]
Dry Clam	0.36 ±0.03 [b]	5.18 ±0.16 [b]	0.33 ±0.10 [b]	49.83 ±6.84 [b]	15.66 ±2.93 [b]	11.87 ±0.83 [b]	95.00 ±1.41 [b]
Frozen Fish	0.41 ±0.01 [b]	3.04 ±0.04 [c]	0.59 ±0.26 [c]	53.74 ±5.81 [b]	44.25 ±4.31 [c]	12.17 ±1.11 [b]	91.00 ±1.41 [c]
Salted Fish	0.23 ±0.04 [c]	5.44 ±0.21 [d]	0.32 ±0.10 [b]	38.35 ±2.35 [c]	4.16 ±0.65 [d]	8.73 ±0.84 [c]	121.00 ±2.19 [db]
SCF 1*	1.01 ±0.09 [d]	1.27 ±0.22 [e]	1.45 ±0.11 [d]	85.20 ±8.38 [d]	101.38 ±3.39 [e]	24.30±2.84 [d]	54.00 ±1.41 [e]
SCF 2*	1.03 ±0.06 [d]	1.17 ±0.10 [e]	1.52 ±0.18 [d]	81.52 ±8.67 [d]	99.18 ±3.51 [e]	24.51 ±2.82 [d]	52.00 ±2.00 [f]
SCF 3*	1.00 ±0.08 [d]	1.21 ±0.14 [e]	1.46 ±0.10 [d]	82.54 ±4.55 [d]	103.07 ±2.20 [e]	25.93 ±3.02 [d]	53.00 ±1.41 [ef]

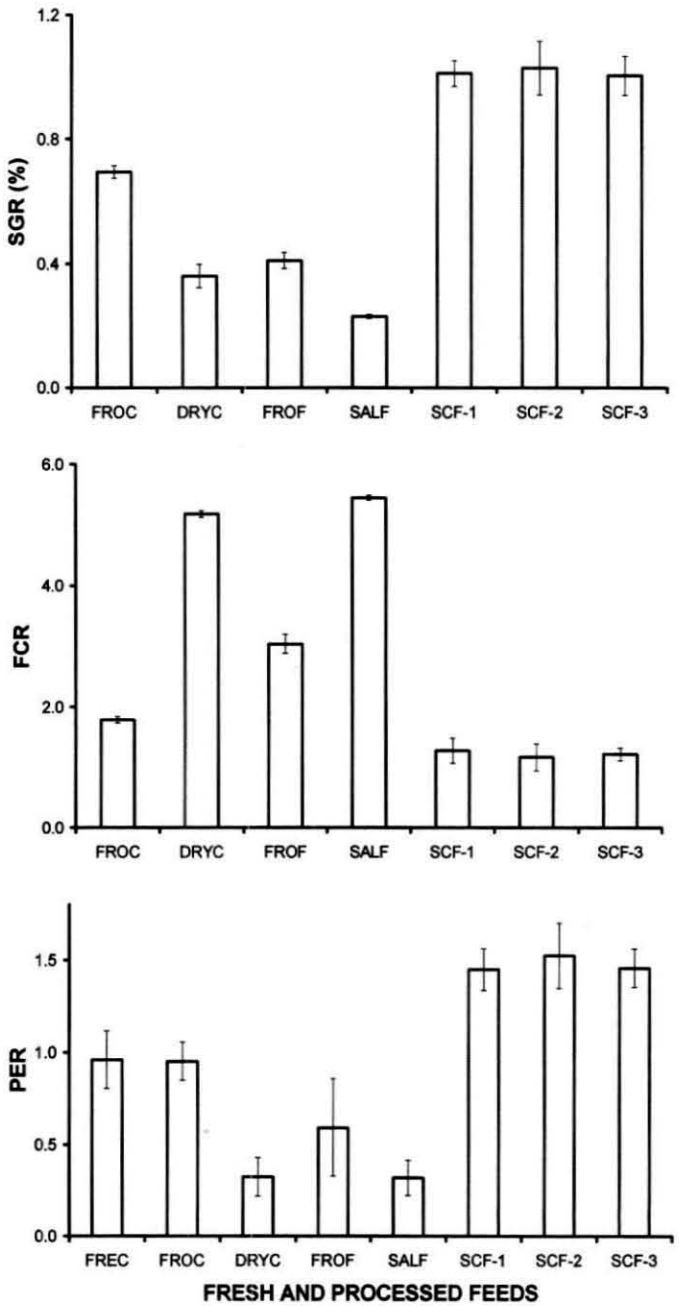
- * SCF-1, SCF-2 and SCF-3 are combination diets containing shrimp head, clam meat and oil sardine, all frozen, at 1:2:7, 1:4:5 and 1:5:4 respectively
- Values in the column with different superscript are significantly ($P<0.05$) different

Figure-7: Percentage gain in wet weight, dry weight and carapace width of juvenile *Scylla serrata* fed fresh and processed feeds



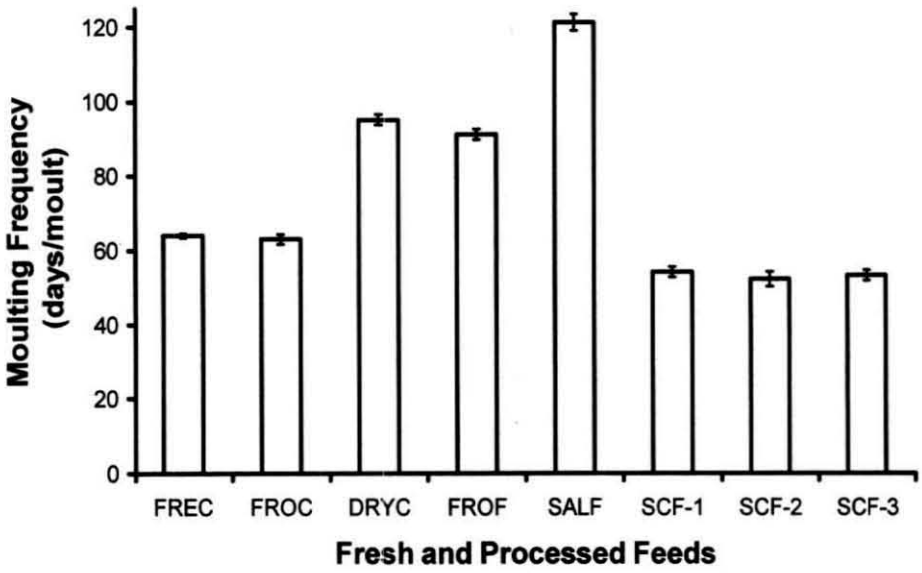
- FREC-fresh clam, FROC-frozen clam, DRYC-dry clam, FROF-frozen fish, SALF-salted fish.
- SCF-1, SCF-2 and SCF-3 are combination diets containing shrimp head, clam meat and oil sardine, all frozen, at 1:2:7, 1:4:5 and 1:5:4 respectively.

Figure-8: SGR, FCR and PER of juvenile *Scylla serrata* fed fresh and processed feeds



- FREC-fresh clam, FROC-frozen clam, FROF-frozen fish, SALF-salted fish,
- SCF-1, SCF-2 and SCF-3 are combination diets containing shrimp head, clam meat and oil sardine, all frozen, at 1:2:7, 1:4:5 and 1:5:4 respectively

Fig-9: Moulting frequency of juvenile Scylla serrata fed fresh and processed feeds.



- FREC-fresh clam, FROC-frozen clam, FROF-frozen fish, SALF-salted fish.
- SCF-1, SCF-2 and SCF-3 are combination diets containing shrimp head, clam meat and oil sardine, all frozen, at 1:2:7, 1:4:5 and 1:5:4 respectively

clam (M=74.98-75.16%, DM=24.84-25.02%, P>0.05), dry clam (M=78.98%, DM=21.02%, P<0.05) and the highest moisture content and lowest dry matter content were observed in salted fish (M=79.28%, DM=20.72%, P<0.05).

4.2.6.2. Crude protein

The highest crude protein content (Table-31, Fig. 11) was found in crabs fed the combination diets (11.45-11.56%, P>0.05) followed by fresh and frozen clam diets (10.28-10.36%, P>0.05), dry clam and frozen fish (8.06-8.34%, P>0.05) and the lowest crude protein was recorded in crabs fed salted trash fish (6.17%, P<0.05).

4.2.6.3. Crude lipid

The highest crude lipid deposition (Table-31, Fig.11) was found in juvenile crabs fed frozen fish (3.69 %, P<0.05) followed by salted fish (3.43, P<0.05), combination diets (1.53-1.68%, P>0.05), fresh, frozen and dry clam (1.32-1.44%, P>0.05). The crude lipid content of crabs fed the fresh and frozen clam was not significantly different (P>0.05) SCF-1 and SCF-3.

4.2.6.4. Crude fiber

The juvenile crabs fed the combination diets (Table-31, Fig.12) had the highest crude fiber content (4.06-4.91, P<0.05) followed by crabs fed the clam feeds and frozen fish (3.26-3.65%, P>0.05), and the lowest crude fibre content was recorded in crabs fed with salted fish (2.90%, P<0.05).

4.2.6.5. Crude ash

The highest crude ash level (Table-31, Fig.12) was observed in crabs fed the combination diets (10.46-10.75, P>0.05), followed by frozen fish (8.44%, P<0.05), fresh, frozen and dry clam (6.23-6.79%, P>0.05) and the lowest level was found in crabs fed the salted fish (5.11%, P<0.05)

4.2.6.6. Nitrogen free extract (NFE)

The NFE content (Table-31, Fig.11) of the crabs ranged from 1.68-3.28 % with relatively high levels in crabs fed fresh clam and salted fish (3.11-3.28%, $P>0.05$) followed by frozen clam (2.64%, $P<0.05$), dry clam, frozen fish and the combination feeds (1.68-1.87%, $P>0.05$).

4.2.6.7. Gross energy

The estimated gross energy content (Table-31, Fig.12) was relatively higher for the crabs fed the frozen fish and the combination diets (3.62-3.67 kJ/g, $P>0.05$) followed by fresh and frozen clam (3.46-3.56 kJ/g, $P>0.05$) and the crabs fed salted fish and dry clam had the lowest energy contents (2.03-2.81 kJ/g, $P<0.05$).

4.2.7. Apparent digestibility

Significantly ($P<0.05$) high apparent digestibility coefficient (Table-32) was observed in crabs fed the fresh and frozen clam and the combination feeds (89.37-91.11%, $P>0.05$) followed by frozen fish (76.53%, $P<0.05$) and the lowest (58.64-60.24%, $P>0.05$) was observed in crabs fed dry clam and salted fish.

4.2.8. Multiple linear regression (MLR) analysis

4.2.8.1. Influence of proximate profile of feeds

MLR analysis of proximate components on the growth responses (Table-33) showed that, SGR, FCR and PER were greatly influenced by the crude fiber content in the combination diets.

4.2.8.2. Influence of amino acids profile of feeds

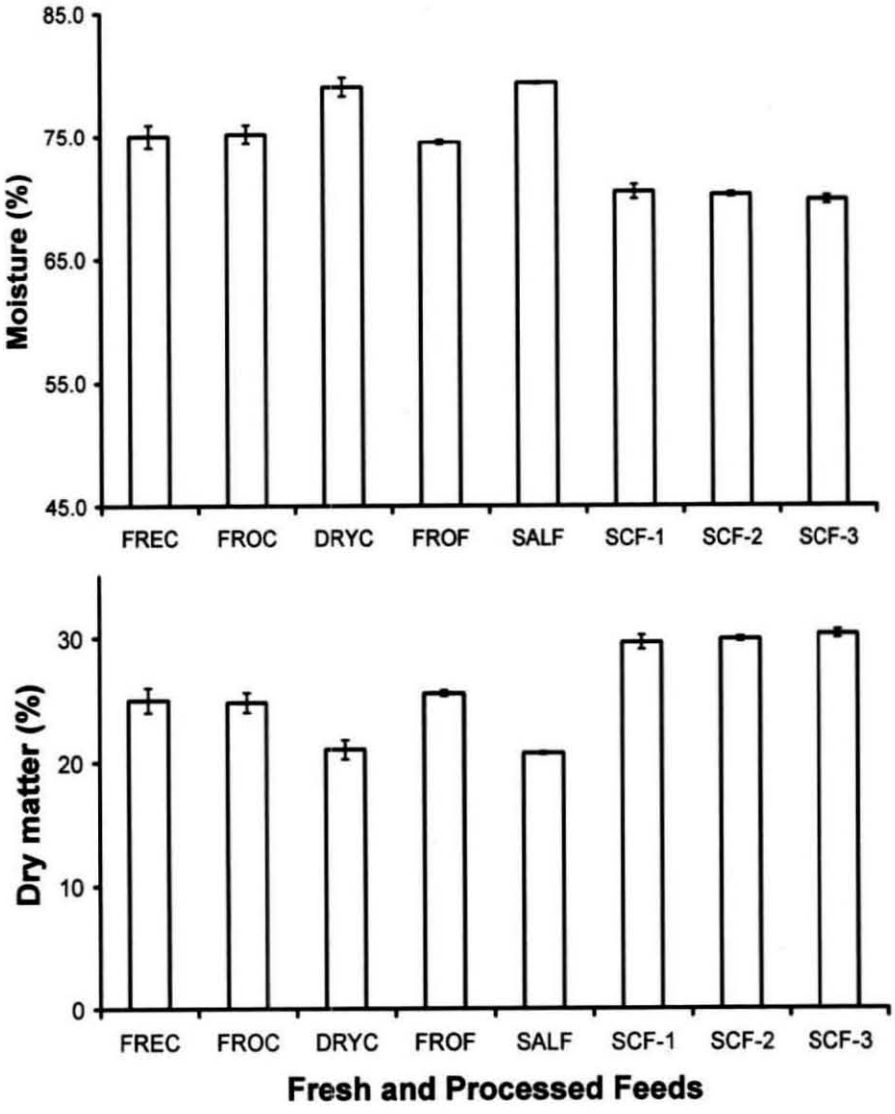
All the 10 essential amino acids were found to influence the growth parameters (SGR - $R^2 = 0.938$, FCR- $R^2 = 0.886$, PER- $R^2 = 0.932$) with the degrees of influence shown in Table-34. Among the non-essential amino acids (Table-35) the SGR was affected ($R^2 = 0.913$) by proline and aspartic acid, where as FCR ($R^2 = 0.764$) by proline and tyrosine and in the case of PER the predictors were found to be proline and cystine ($R^2 = 0.881$).

Table-31: Proximate profile of the juvenile *Scylla serrata* fed fresh and processed feeds (in % as such basis)

Feeds	Moisture	Dry matter	Crude protein	Crude lipid	Crude fiber	Crude ash	Nitrogen free extract	Gross energy (kJ/g)
Fresh clam	74.98 ±0.97^{a}	25.02 ±0.97^{a}	10.28 ±0.16^{a}	1.44 ±0.10^{ab}	3.64 ±0.13^{ac}	6.38 ±1.01^{a}	3.28 ±0.10^{a}	3.56 ±0.02^{ab}
Frozen clam	75.16 ±0.79^{a}	24.84 ±0.79^{a}	10.36 ±0.13^{a}	1.41 ±0.06^{ab}	3.65 ±0.15^{abc}	6.79 ±0.94^{a}	2.64 ±0.06^{b}	3.46 ±0.04^{b}
Dry clam	78.98 ±0.78^{b}	21.02 ±0.77^{b}	8.34 ±0.23^{b}	1.32 ±0.08^{a}	3.26 ±0.29^{a}	6.23 ±0.61^{a}	1.87 ±0.08^{c}	2.81 ±0.09^{c}
Frozen fish	74.45 ±0.21^{a}	25.55 ±0.21^{a}	8.06 ±0.20^{b}	3.69 ±0.08^{c}	3.58 ±0.12^{a}	8.44 ±1.31^{b}	1.78 ±0.08^{c}	3.67 ±0.02^{a}
Salted fish	79.28 ±0.10^{b}	20.72 ±0.10^{b}	6.17 ±0.18^{c}	3.43 ±0.13^{d}	2.90 ±0.12^{b}	5.11 ±0.10^{c}	3.11 ±0.13^{a}	2.03 ±0.06^{d}
SCF 1*	70.39 ±0.58^{d}	29.61 ±0.58^{d}	11.46 ±0.84^{d}	1.53 ±0.15^{abe}	4.06 ±0.34^{cd}	10.75 ±1.43^{d}	1.81 ±0.15^{c}	3.62 ±0.11^{d}
SCF 2*	70.12 ±0.18^{d}	29.88 ±0.18^{d}	11.45 ±0.69^{d}	1.68 ±0.04^{e}	4.41 ±0.37^{e}	10.65 ±0.96^{d}	1.68 ±0.10^{c}	3.66 ±0.08^{a}
SCF 3*	69.71 ±0.33^{d}	30.29 ±0.33^{d}	11.56 ±0.81^{d}	1.56 ±0.23^{be}	4.91 ±0.26^{f}	10.46 ±1.14^{d}	1.79 ±0.18^{c}	3.65 ±0.08^{a}

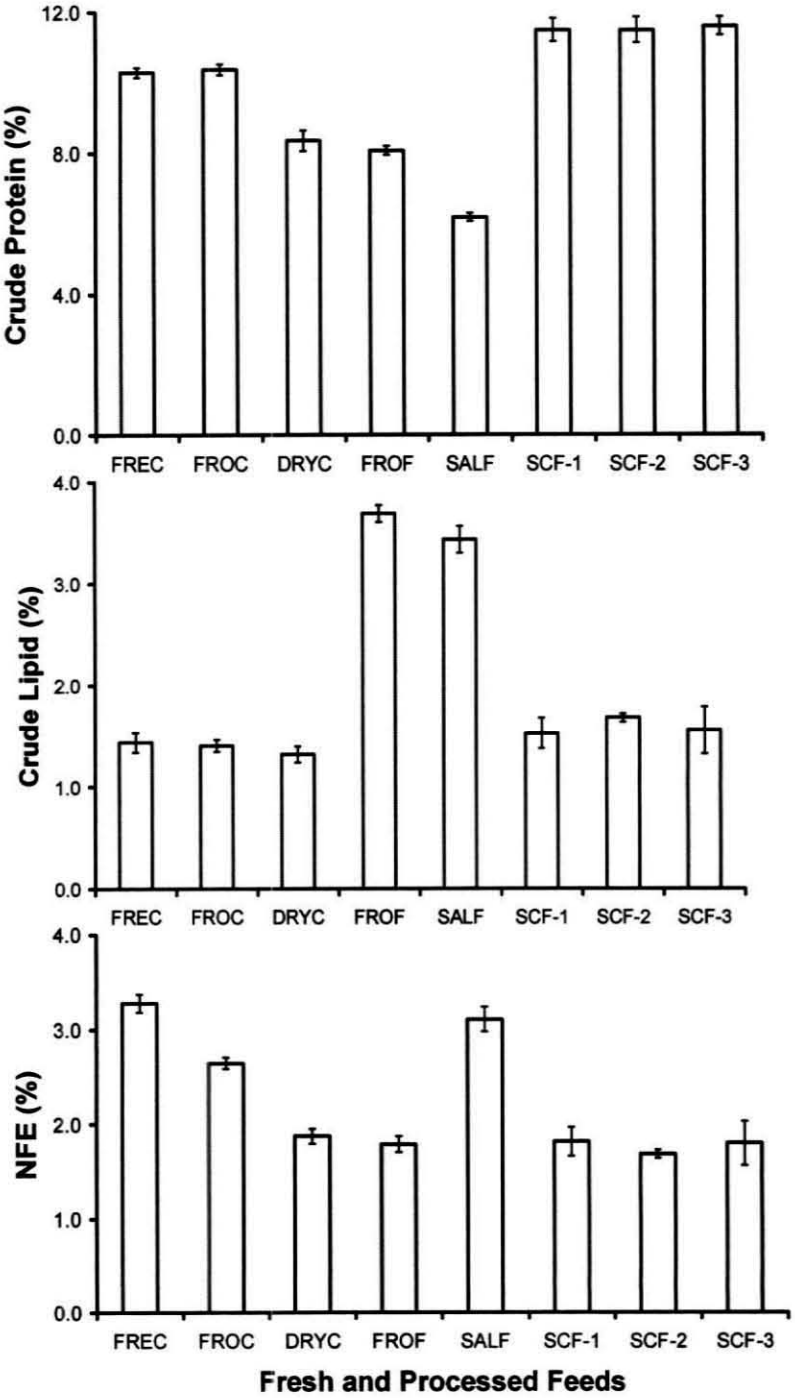
- * SCF-1, SCF-2 and SCF-3 are combination diets containing shrimp head, clam meat and oil sardine, all frozen, at 1:2:7, 1:4:5 and 1:5:4 respectively
- Means with different superscript letters are significantly different ($P<0.05$).

Figure-10: Percentage moisture and dry matter contents of juvenile *Scylla serrata* fed fresh and processed feeds (as such basis)



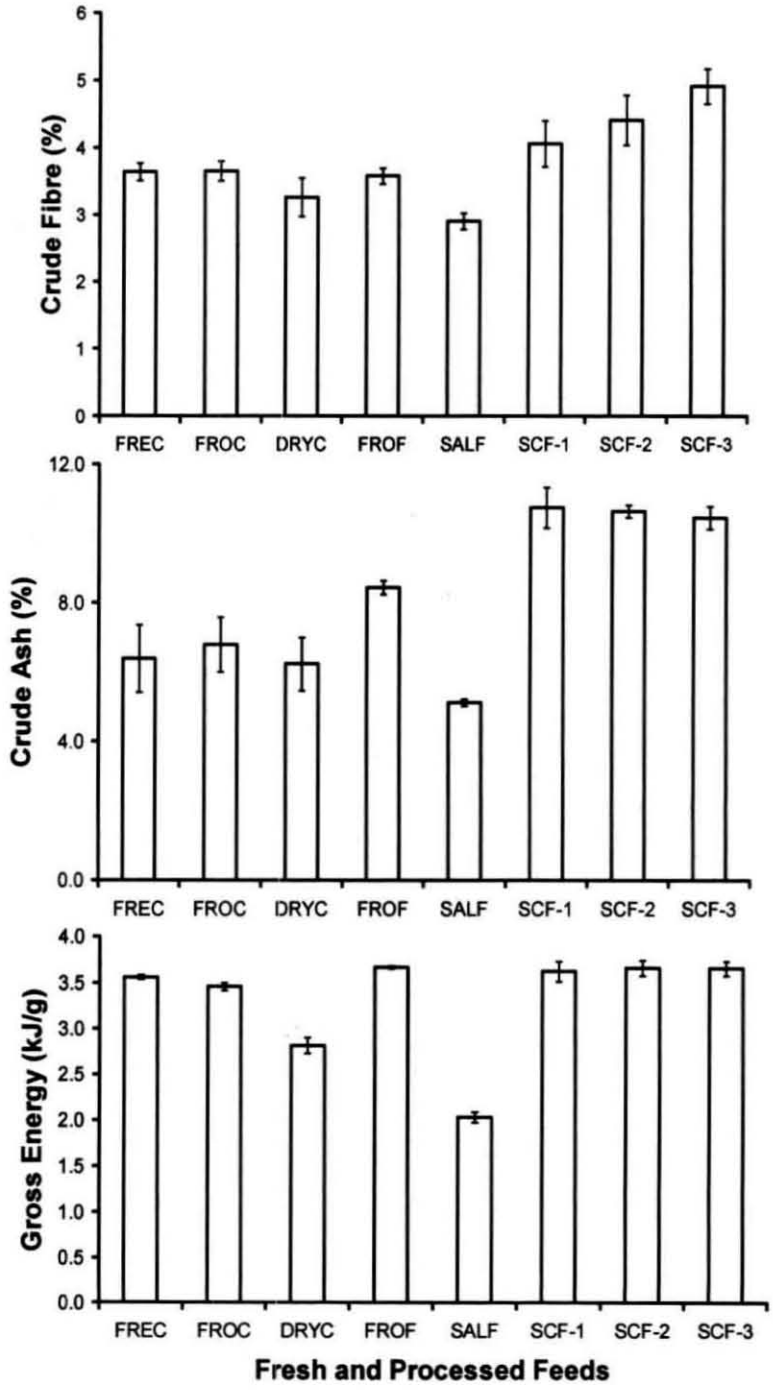
- FREC-fresh clam, FROC-frozen clam, FROF-frozen fish, SALF-salted fish,
- SCF-1, SCF-2 and SCF-3 are combination diets containing shrimp head, clam meat and oil sardine, all frozen, at 1:2:7, 1:4:5 and 1:5:4 respectively

Figure-11: Crude protein, crude lipid and nitrogen free extract (NFE) contents of juvenile *Scylla serrata* fed fresh and processed feeds (as such basis).



- FREC-fresh clam, FROC-frozen clam, DRYC-dry clam, FROF-frozen fish, SALF-salted fish.
- SCF-1, SCF-2 and SCF-3 are combination diets containing shrimp head, clam meat and oil sardine, all frozen, at 1:2:7, 1:4:5 and 1:5:4 respectively.

Figure-12: Crude fibre, crude ash and gross energy contents of juvenile *Scylla serrata* fed fresh and processed feeds (as such basis).



- FREC-fresh clam, FROC-frozen clam, DRYC-dry clam, FROF-frozen fish, SALF-salted fish
- SCF-1, SCF-2 and SCF-3 are combination diets containing shrimp head, clam meat and oil sardine, all frozen, at 1:2:7, 1:4:5 and 1:5:4 respectively.

Table-32: Apparent digestibility coefficients (ADC) shown by juvenile *Scylla serrata* fed fresh and processed feeds

FEEDS	ADC (%)	FCR
Fresh Clam	91.11 ±5.67[a]	1.76 ±0.05 [a]
Frozen Clam	90.97 4.61[a]	1.78 ±0.06 [a]
Dry Clam	60.24 ±1.64[b]	5.18 ±0.16 [b]
Frozen Fish	76.53 ±4.14[c]	3.04 ±0.04 [c]
Salted Fish	58.64 ±1.89[b]	5.44 ±0.21 [d]
SCF 1*	89.37 ±4.39[a]	1.27 ±0.22 [e]
SCF 2*	89.65 ±4.05[a]	1.17 ±0.10 [e]
SCF 3*	90.25 ±4.55[a]	1.21 ±0.14 [e]

- * SCF-1, SCF-2 and SCF-3 are combination diets containing shrimp head, clam meat and oil sardine, all frozen, at 1:2:7, 1:4:5 and 1:5:4 respectively
- Means with different superscript letters are significantly different (*P<0.05*).

4.2.8.3. Influence of fatty acids profile of feeds

Among the saturates 16:0 (Table-36, 37 and 38) showed a relatively low level of influence on FCR (R^2 = 0.546), but did not show any influence on SGR or PER. Among the MUFAs 17:1n6, 20:1n11 and 16:1n7 were found to influence SGR (R^2 = 0.925), where as FCR was affected by 17:1n6, 20:1n11 and 24:1n3 (R^2 = 0.842) and PER by 17:1n6 and 20:1n11 (R^2 = 0.764). Among the PUFAs, arachidonic acid and linoleic acid have influenced the SGR (R^2 = 0.710), while PER was affected by all the PUFAs, at a lower degree (R^2 = 0.578). FCR did not show any relationship with PUFAs. Among the n3 faty acids, 22:6n3, 20:5n3 and 24:1n3 had influence (R^2 = 0.879) on SGR, whereas 22:6n3, 20:5n3, 24:1n3, and 18:3n3 (R^2 = 0.857) had influence on PER. Among n6 fatty acids, 20:4n6 and 17:1n6 were found to influence the SGR (R^2 = 0.892) and PER (R^2 = 0.770) where as FCR was influenced by 20:4n6 and 18:2n6 (R^2 = 0.878). Both the n3 HUFAs, viz., 22:6n3 and 20:5n3 were found to be predictors of SGR (R^2 = 0.736), FCR (R^2 = 0.942) and PER (R^2 = 0.707).

4.3. Energy budgeting

4.3.1. Gross nutritional profile of the feed

The crude protein and crude lipid contents of the freshly shucked meat of black clam *Villorita cyprinoides* used for the energy budgeting experiments were 59.01 ±1.07 % and 9.01 ±0.81 % on dry matter basis, respectively. The crude ash level was 5.25 ±0.49 %, where as nitrogen free extract was 26.74 ±1.13% of the dry meat. The gross energy value of the clam meat was estimated to be 22.11 ±0.98 kJ/g dry matter and the P/E ratio was 26.69 ±1.08 mg protein /kJ. The crude fiber content was found to be negligible (<0.001 %).

4.3.2. Survival

Cent percent survival of juvenile crabs was recorded in the experiment.

4.3.3. Growth

The growth performance of the crabs during the experiment is given in Table-39. Juvenile crabs expressed a gain of 73.82 % in wet wt. and 55.27 of % dry wt.

Table-33: Multiple linear regression analysis of proximate profile vs. SGR, FCR and PER of fresh and processed feeds

Dependent factors	R	R Square	Adjusted R Square	Std. Error of the estimate	Change Statistics				
					R Square change	F change	df1	df2	Sig. F change
SGR	0.955	0.912	0.893	0.109	0.912	49.052	4	19	0.000
FCR	0.876	0.767	0.717	1.098	0.767	15.595	4	19	0.000
PER	0.932	0.869	0.842	0.194	0.869	31.616	4	19	0.000
PREDICTOR(S)									
SGR	CF > NFE > CP > CA								
FCR	CF > CA > CP > NFE								
PER	CF > NFE > CP > CA								

Table-34: Multiple linear regression of essential amino acid profile vs. SGR, FCR and PER of fresh and processed feeds

Independent factor groups	R	R Square	Adjusted R Square	Std. Error of the estimate	Change Statistics				
					R Square change	F change	df1	df2	Sig. F change
SGR	0.968	0.938	0.89	0.111	0.938	19.528	10	13	0.000
FCR	0.941	0.886	0.799	0.9256	0.886	10.146	10	13	0.000
PER	0.965	0.932	0.879	0.1693	0.932	17.76	10	13	0.000
PREDICTOR(S)									
SGR	All the essential amino acids								
FCR	" "								
PER	" "								

Table-35: Multiple linear regression of non-essential amino acid profile vs. SGR, FCR and PER of fresh and processed feeds

Independent factor groups	R	R Square	Adjusted R Square	Std. Error of the estimate	Change Statistics				
					R Square change	F change	df1	df2	Sig. F change
SGR	0.956	0.913	0.905	0.103	0.251	60.651	1	21	0.000
FCR	0.874	0.764	0.741	1.0506	0.349	31.045	1	21	0.000
PER	0.938	0.881	0.869	0.1761	0.217	38.176	1	21	0.000
PREDICTOR(S)									
SGR	Pro > Asp								
FCR	Pro > Tyr								
PER	Pro > Cys								

Table-36: Multiple linear regression analysis of fatty acid profile vs. SGR of fresh and processed feeds

Independent factor groups	R	R Square	Adjusted R Square	Std. Error of the estimate	Change Statistics				
					R Square change	F change	df1	df2	Sig. F change
Saturated	0.478	0.228	0.193	0.3	0.228	6.513	1	22	0.018
MUFA	0.962	0.925	0.913	9.84E-02	0.064	17.079	1	20	0.001
PUFA	0.843	0.71	0.683	0.1881	0.461	33.451	1	21	0.000
n3	0.938	0.879	0.861	0.1246	0.143	23.67	1	20	0.000
n6	0.944	0.892	0.882	0.1149	0.612	119.042	1	21	0.000
n3 HUFA	0.858	0.736	0.711	0.1796	0.428	34.053	1	21	0.000
PREDICTOR(S)									
Saturated	C16:0								
MUFA	17:1n6, 20:1n11 and 16:1n7								
PUFA	20:4n6, 18:2n6								
n3	22:6n3, 20:5n3, 24:1n3								
n6	20:4n6, 17:1n6								
n3 HUFA	22:6n3, 20:5n3,								

Table-37: Multiple linear regression analysis of fatty acid profile vs. FCR of fresh and processed feeds

Independent factor groups	R	R Square	Adjusted R Square	Std. Error of the estimate	Change Statistics				
					R Square change	F change	df1	df2	Sig. F change
Saturated	0.739	0.546	0.525	1.4228	0.546	26.439	1	22	0.000
MUFA	0.918	0.842	0.819	0.8791	0.069	8.808	1	20	0.008
PUFA	0.758	0.574	0.485	1.482	0.574	6.412	4	19	0.002
n3	No								
n6	0.87	0.757	0.734	1.0655	0.431	37.204	1	21	0.000
n3 HUFA	0.971	0.942	0.937	0.5189	0.524	190.692	1	21	0.000
PREDICTOR(S)									
Saturated	C16:0								
MUFA	17:1n6, 20:1n11 and 24:1n3								
PUFA	No influence								
n3	No influence								
n6	No influence								
n3 HUFA	22:6n3, 20:5n3,								

Table-38: Multiple linear regression analysis of fatty acid profile vs. PER of fresh and processed feeds

Independent factor groups	R	R Square	Adjusted R Square	Std. Error of the estimate	Change Statistics				
					R Square change	F change	df1	df2	Sig. F change
Saturated	0.443	0.196	0.16	0.4468	0.196	5.374	1	22	0.030
MUFA	0.874	0.764	0.742	0.2476	0.537	47.871	1	21	0.000
PUFA	0.760	0.578	0.489	0.3486	0.578	6.493	4	19	0.002
n3	0.926	0.857	0.827	0.203	0.057	7.499	1	19	0.013
n6	0.878	0.77	0.749	0.2444	0.541	49.53	1	21	0.000
n3 HUFA	0.841	0.707	0.679	0.276	0.394	28.271	1	21	0.000
PREDICTOR(S)									
Saturated	C16:0								
MUFA	17:1n6 and 20:1n11								
PUFA	All PUFAs								
n3	22:6n3, 20:5n3, 24:1n3, 18:3n3								
n6	20:4n6, 17:1n6								
n3 HUFA	22:6n3, 20:5n3,								

with a SGR of 0.89 %. FCR and PER recorded in the experiment were 1.5 and 0.98 respectively. Carapace width gain was recorded as 15.86 % with an average intermoult duration of 63 days.

4.3.4. Proximate composition of juvenile crabs

The proximate composition (Table-40) of juvenile crabs showed 25.38 % dry matter, with 10.90 % crude protein, 1.44 % crude lipid, and 3.73 % of nitrogen free extract. The crude fibre and ash contents were 3.21 % and 5.67 % respectively. The gross energy content was estimated as 3.97 kJ/ g wet weight.

4.3.5. Feeding rate

The energy intake was calculated from the feed intake data obtained during the experimental period and the results (Table-41) were expressed as joules (J) per g live mid body weight of the crab per day. Approximately 75.27 ±1.53 % of the total feed offered was consumed by the experimental crabs which correspond to 203.08 ±5.24 J/g/day. About 195.51 ±8.86 J/g/day was assimilated with an assimilation efficiency of 96.27 ±0.62%.

The energy intake rate at different moult stages, viz., intermoult, pre-moult and post-moult stages was calculated from the daily feed intake data. Juvenile crabs exhibited significant (*P>0.05*) difference in the rate of energy intake at various moult stages. The highest feeding rate was seen during the intermoult stage (272.46±1.02) and the feeding intensity was found to decrease at pre-moult (54.94±0.69 J/g/day) and post-moult (183.13 ±0.89 J/g/day) stages.

4.3.6. Conversion rate

From the Table-41, it is evident that the juvenile crabs converted about 9.32 ±0.21% (18.41 ±081 J/g/day) of the total intake energy for production or growth (P), and the formation of the moult (E) required 7.94 ±0.08% (15.69 ±0.08 J/g/day) of the total intake energy,. The energy expenditure towards moult production was considered as energy loss, since the moult shell is discarded fully at the time of

Table-39: The growth response of juvenile Scylla serrata fed freshly shucked clam meat

SGR	0.89 ±0.05
FCR	1.50 ±0.03
PER	0.98 ±0.11
% WET WT. GAIN	73.82 ±1.82
% DRY WT. GAIN	55.27 ±4.37
% CW GAIN	15.86 ±0.51
Inter moult duration (days/ moult)	62.67 ±1.63

Table-40: The proximate profile of juvenile *Scylla serrata* fed freshly shucked clam meat (% as such basis)

Moisture	74.62 ±1.01
Dry matter	25.38 ±1.01
Crude protein	10.90 ±0.21
Crude lipid	1.44 ±0.09
Crude fiber	3.21 ±0.20
Crude ash	5.63 ±0.85
Nitrogen free extract	3.73 ±0.10
Gross energy	3.97 ±0.13 kJ/g

ecdysis. The gross energy conversion rate is the sum total of growth rate and moult production rate (P+E) and therefore accounted for 34.10 ±0.76 J/g/day with a gross conversion efficiency of 17.26 ±0.84% of the total intake energy. At 96.45 % assimilation efficiency (190.56 ±8.86 J/g/day of total intake energy), 17.89 ±0.79% net conversion efficiency was achieved.

4.3.7. Excretion rate

About 7.01 ±0.18 J/g/day (3.54 % of intake energy; Table- 41) was lost as excretory products of metabolism and as faecal matter. The rate of faecal loss was about 1.83 % (3.62 ±0.25 J/g/day) and urinary loss was about 1.71 % (3.39 ±0.31 J/g/day) of the total intake energy. About 51.64 % of the total excretory loss was accounted by the faeces and remainder (48.36 %) was contributed by the urinary loss principally accounted by ammonia excretion.

4.3.8. Metabolic rate

4.3.8.1. Calculated total metabolic rate

Taking consideration of the energy intake (C), production (growth, P and moult, E) and excretion (faecal loss, F and urinary loss, U) the metabolism was calculated using the equation M= C- P + E + F+U. The total metabolic rate of the juvenile crabs, was calculated as 156.46 ±1.83 J/g/day, which was about 79.20 ±1.01 % of the total intake energy (Table-41).

4.3.8.2. Estimated metabolic rate

Metabolic rate was estimated by quantifying the routine or basal oxygen uptake and the oxygen uptake associated with the apparent specific dynamic action. Also, the values were corrected for the different moult stages of the juvenile crabs. The oxygen uptake associated with routine metabolism was found to be 0.19 ±0.03 ml/g/hr (89.85 ±1.06 J/g/day) and that with apparent specific dynamic action was 0.14 ±0.03 ml/g/hr (68.45 ±0.86 J/g/day), which corresponds to 45.48 ±0.18% and 34.64 ± 1.31% of the estimated total metabolic rate respectively. The total estimated metabolic rate (Table-42) was 80.12 ±1.08% (158.28 ±0.74 J/g/day or 0.33 ±0.06 ml

Table-41: Feeding, food utilization and growth of juvenile *Scylla serrata*

Energy budgeting indices	J/ g/ day	% of total energy intake
Energy intake	197.57 ±5.24	
Assimilation rate (AR)	190.56 ±8.86	
Growth rate (P)	18.41 ± 0.81	9.32
Moult loss (E)	15.69 ±0.08	7.94
Conversion rate (CR=P+E)	34.10 ±0.76	17.26
Faecal energy (F)	3.62 ±0.25	1.83
Urinary energy (U)	3.39 ±0.31 (=0.0069 mg/g/hr total NH_3)*	1.71
Excretion rate (E=F+U)	7.01 ±0.18	3.54
Metabolic rate (M) (calculated)	156.46 ±1.83	79.2
Assimilation efficiency (AE)	96.45 ±0.62 %	
Gross conversion efficiency (GCE)	17.26 ±0.84 %	
Net conversion efficiency (NCE)	17.89 ±0.79 %	

* Energy equivalent of 1 mg NH_3 is equivalent to 20.5 J (Brafield, 1995)

Table-42: Estimated rates of standard metabolic rate, specific dynamic action and total metabolism

Metabolic rates (estimated)	O_2 ml/g/hr	Energy values J/g/day
Standard metabolic rate (SMR)	0.19 ±0.03	89.85 ±1.06
Apparent specific dynamic action (ASDA)	0.14 ±0.03	68.45 ±0.86
Metabolism (M = RM + ASDA)	0.33 ±0.06	158.28 ±1.99

- Energy equivalent of 1 ml O_2 is equivalent to 20.098 J (Engelman, 1966)

O_2 /g/hr) of the total intake energy. The estimated total metabolic rate was about 1.16 % higher than that of the calculated total metabolic energy. The difference may be accounted for by the animal associated parameters such as handling stress.

4.3.8.3. Changes in the metabolic rate associated with moult stages

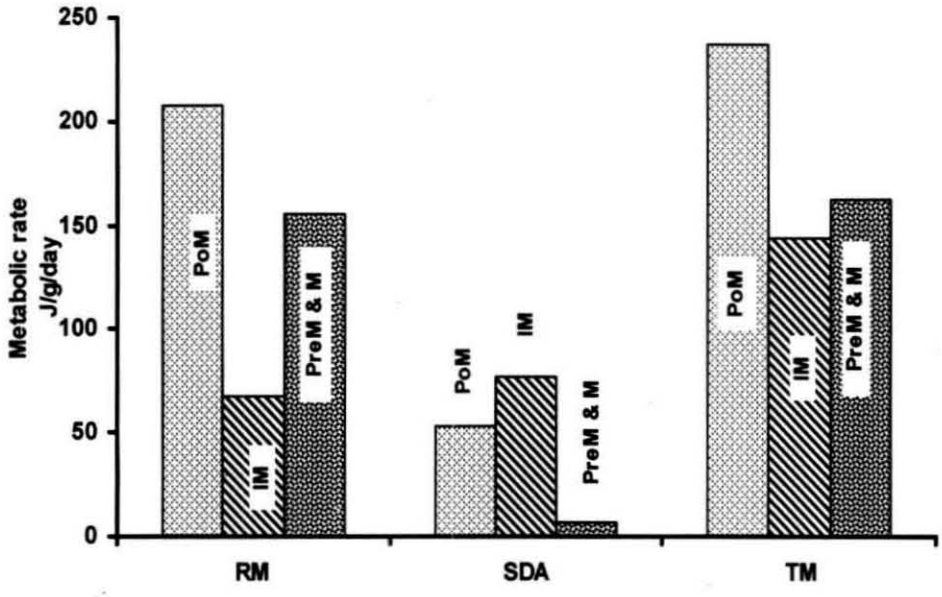
To get the corrected metabolic rate the respirometric estimations were made three times under the assumption that considerable variations in the physiological state of the crabs occur during the various stages of the moult cycle (Passano, 1960). The routine metabolic rate (Table 43; Fig.13) was highest at moult and post-moult stages (207.89 ±0.42 J/g/day, 88.00 ±0.16 % of MR), followed by pre-moult stage (155.17 ±0.21 J/g/day, 95.66 ±0.14 % of total MR) and the lowest was observed at intermoult stage (67.20 ±0.10 J/g/day, 46.68 ±0.12 % of MR). In the case of apparent specific dynamic action (ASDA), the highest was recorded at intermoult stage (76.76 ±0.16 J/g/day, 53.32 ±0.19 % of total MR) corresponding to the peak feeding intensity of the juvenile crabs, and was further followed by the post-moult stage (53.11 ±0.35 J/g/day, 22.48 ±0.11 % of total MR). The lowest SDA was recorded at pre-moult stage (7.04 ±0.15 J/g/day, 4.34 ±0.02 % of total MR), associated with lowering of the feeding intensity. The highest total MR was observed at post-moult and pre-moult stages (236.25 ±0.37 J/g/day, 129.00 ±3.12 % of daily intake energy) followed by pre-moult stage (162.06 ±0.25 J/g/day, 295.27 ±1.93 % of daily intake energy) and the lowest MR at the intermoult stage (143.96 ±0.42 J/g/day, 52.84 ±1.82 % of daily intake energy). From the routine metabolic expenses and SDA it is clear that in juvenile *Scylla serrata*, the intermoult stage was the most stable moult stage. In the pre-moult as well as post-moult stages, there were a phenomenal increase in routine metabolic rates of 230.91% and 309.37 %, respectively than that obtained at intermoult stage. A reverse trend was observed in the case of SDA, in which a reduction of 69.20% and 9.18% for pre-moult stage and moult and post-moult stages occurred as compared to the intermoult stage.

Table-43: Estimates of standard metabolism, specific dynamic action and total metabolism, of juvenile *Scylla serrata* during various moult stages

	Moult and post-moult stage	Intermoult stage	Pre-moult stage
Intake energy (IE)	183.13 ±0.89 [a] (n=6)	272.46±1.02 [b] (n=6)	54.94±0.69 [b] (n=4)
Routine metabolism (RM)	207.89 ±0.42 [a] (n=6)	67.20±0.10 [b] (n=6)	155.17±0.21 [c] (n=4)
RM as % of MR	88.00±0.16 [a] (n=6)	46.68±0.12 [b] (n=6)	95.66±0.14 [c] (n=4)
Apparent Specific Dynamic Action (ASDA)	53.11±0.35 [a] (n=6)	76.76±0.16 [b] (n=6)	7.04±0.15 [c] (n=4)
ASDA as % of MR	22.48±0.11 [a] (n=6)	53.32±0.19 [b] (n=6)	4.34±0.02 [c] (n=4)
Metabolic rate (MR)	261.00±0.37 [a] (n=6)	143.96±0.42 [b] (n=6)	162.21±0.25 [c] (n=4)
MR as % of IE	129.00±3.12 [a] (n=6)	52.84±1.82 [b] (n=6)	295.27±1.93 [c] (n=4)

- The values with different superscript in the column are significantly different ($P<0.05$)
- n - number of observations

Figure-13: Estimated metabolic variation in juvenile *Scylla serrata* at different moult stages



RM - Routine metabolic rate
SDA - Specific dynamic action
TM - Total metabolic rate

PoM - Post-moult stage
IM - Intermoult stage
Pre M & M - Pre-moult and moult stages

4.3.9. Feeding rate and moult cycle

Feeding pattern during the moult cycle (Fig.14) was observed and three significantly ($P<0.05$) different feeding rates were observed corresponding to the post-moult, intermoult and pre-moult stages. Feeding rate was found to increase gradually after the day of moulting, till the next 4 days passing the post-moult stage (0-7.29 ±0.8 %) and reached the maximum (7.54±1.28 %) and stabilised between 5^{th} and 55^{th} days of intermoult period in juvenile crabs. This is followed by an abrupt decline (7.54-0 %) in the 4 days preceding moulting, ie., at the pre-moult stage. The crabs were found to starve the day prior to and about 18-124 hrs after moulting.

4.3.10. Feeding intensity

The observations on feeding intensity (Fig.15) exhibited by the juvenile crabs between 1700 hrs and 0700 hrs, revealed two peak intensities. The first one was observed between 1700 and 1900 hrs with about 50% consumption of the ration followed by near zero intensities between 1900 and 2200 hrs, and further showed a gradual increase of about 4.56 % till 0100 hrs and the next phase of improved feed intake extended till 0500 hrs with the second peak consumption of about 18.82% of the ration at around 0300 hrs. The intensity further declined to reach 1-2% between 0500 and 0600 hrs.

4.3.11. Construct of energy budget

From the results (Table-44; Fig. 16) the total intake energy can be apportioned as follows: 9.32 % of the total intake energy was deposited as growth (P); where as 7.94 % was lost as moult loss (E); about 1.83 % was discarded as faecal loss (F) and about 1.71% was expelled as urinary loss (U). After subtracting the production and losses, it is apparent that 79.20% of the intake energy was accounted by metabolic expenses (calculated metabolic rate, Mc) spend towards routine metabolism and specific dynamic action. The estimation of actual expenses (Fig. 17) on metabolism (Me) gave 45.48 % of routine metabolic (RM) expenditure and 34.64 % of intake energy towards apparent specific dynamic action (ASDA) with a total metabolic expense of 80.12 % against the calculated metabolic expenditure of 79.20%.

Fig-14: Feeding pattern over the moult cycle in juvenile *Scylla serrata*

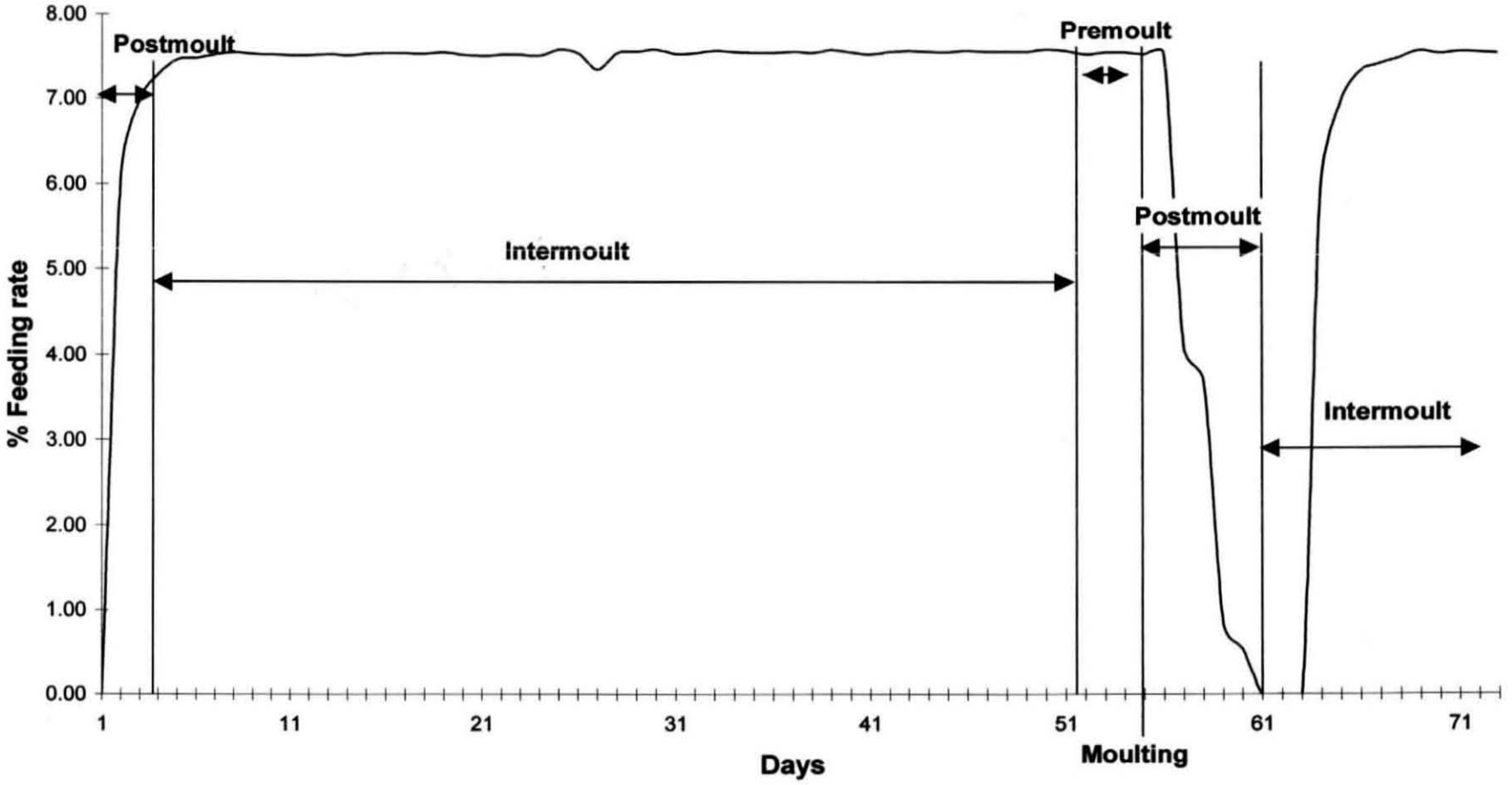


Fig-15: Feeding intensity of juvenile Scylla serrata fed single ration of fresh clam meat between 1700 hrs and 0600 hrs

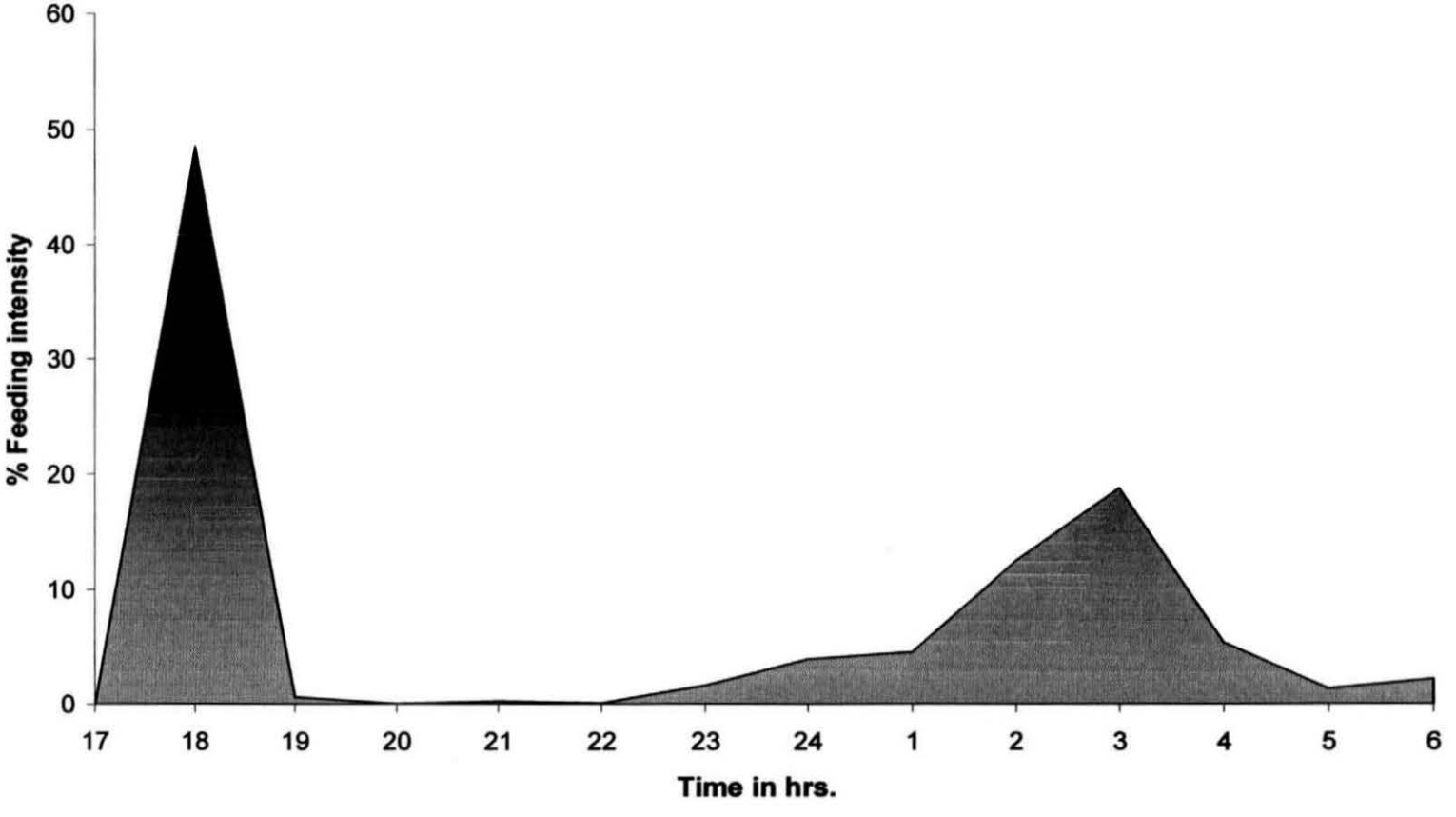


Table-44: Energy budget of juvenile *Scylla serrata* derived as percentage of total food energy consumed

	g/J/day	Percentage of energy intake (IE)
Energy intake (IE)	197.57 ±5.24	100 %
Growth (P)	18.41 ± 0.81	9.32 %
Moult loss (E)	15.69 ±0.08	7.94 %
Faecal energy (F)	3.62 ±0.25	1.83 %
Urinary energy (U)	3.39 ±0.31	1.71 %
Total metabolism calculated (Mc = IE - P+E+F+U)	156.46 ±1.83	79.2 %
Routine metabolism estimated (RM)	89.85 ±0.39	45.48 ±1.08 %
Apparent specific dynamic action estimated (ASDA)	68.44 ±0.51	34.64 ± 1.31 %
Total metabolism estimated (Me = RM + ASDA)	158.28 ±0.74	80.12 ±2.19 %

Fig-16: Construct of energy budget and food utilisation in juvenile Scylla serrata (using calculated metabolism, values expressed as % of intake energy)

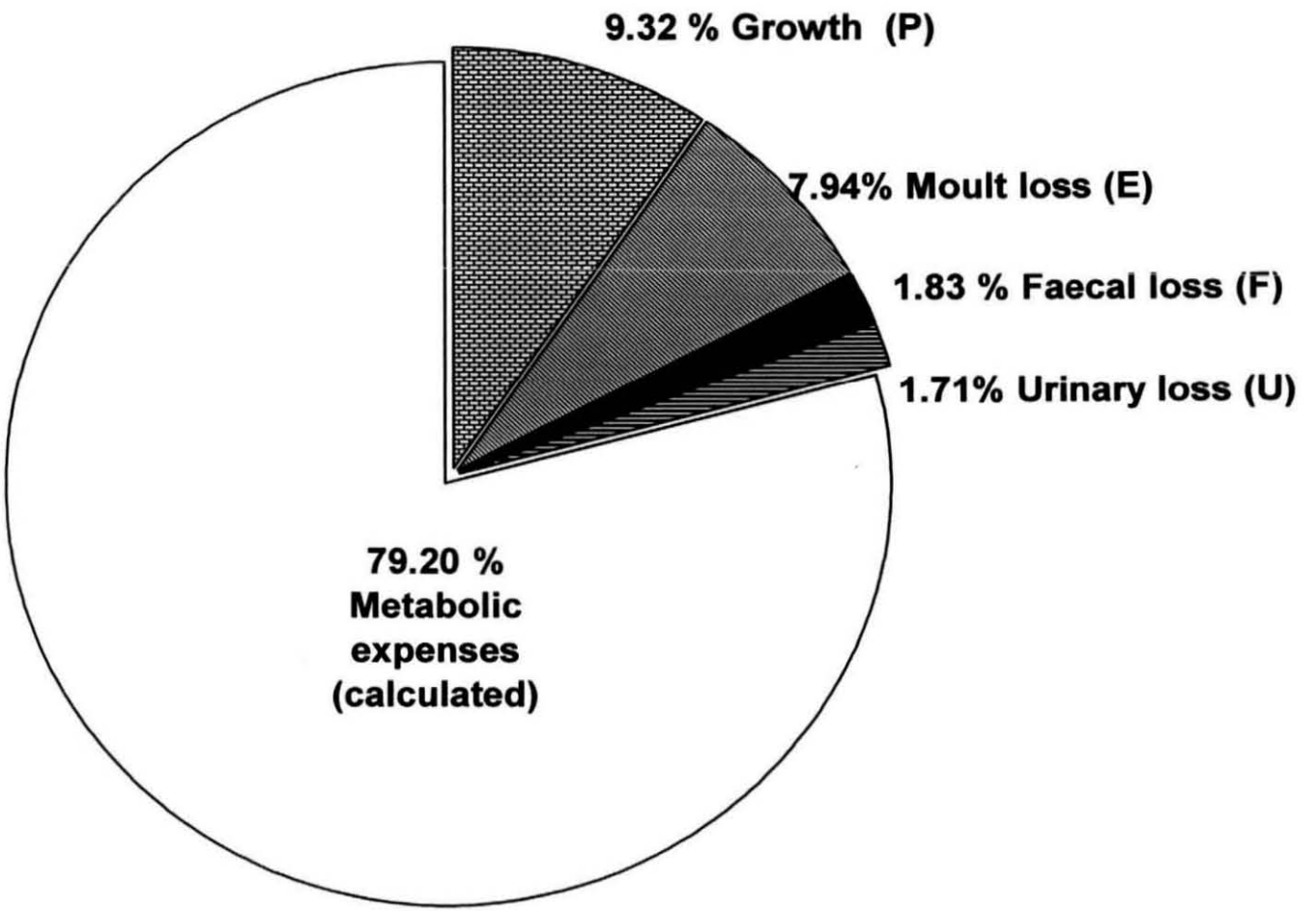
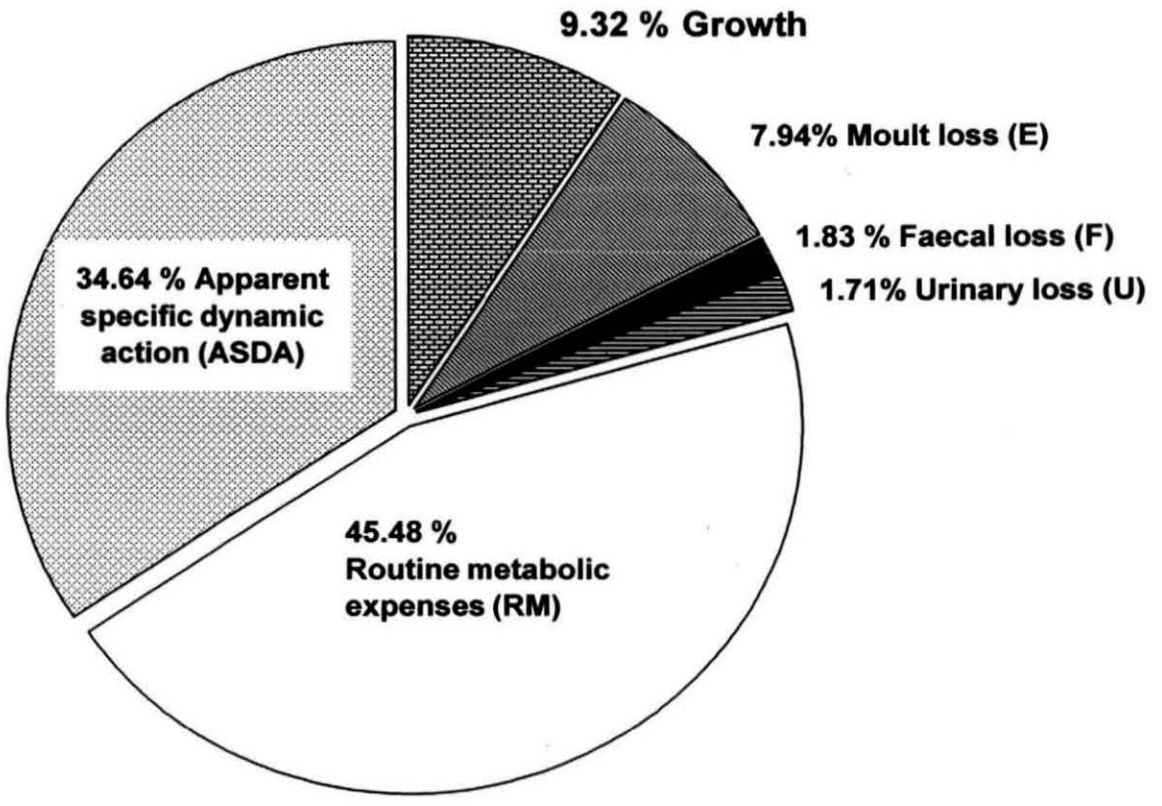


Fig-17: Construct of energy budget and food utilisation in juvenile *Scylla serrata* (using estimated metabolism; values expressed as % of intake energy)



4.4. Dietary protein requirement

4.4.1. Amino acids profile of diets

The amino acid profiles of the formulated diets having graded levels of protein are shown in Table 45. The diets had an essential amino acid to non-essential amino acid ratio of 0.87.

4.4.2. Survival

The dietary protein levels had significant ($P<0.05$) influence on the survival resulting in 100 % mortality of the crabs fed low protein diets, CP-15 (15% crude protein) and CP-20 (20% crude protein) during the first moulting itself. All other diets showed cent percent survival. The crabs fed lower protein diets were unable to moult fully and the dead crabs were seen in a partly moulted condition.

4.4.3. Growth response

Since crabs in treatment CP-15 and CP-20 suffered 100% mortality, the growth parameters were recorded for the remaining treatments (CP-25, 30, 35, 40, 45 and 50). SGR (%/day), FCR, PER, wet and dry wt. gain (% of initial weight), carapace width gain (% of initial carapace width) and moulting frequency (days/moult) were taken as growth parameters to assess the effectiveness of the feeds.

4.4.3.1. Weight gain

The weight gain (Table- 46, Fig. 18) was found to be significantly ($P<0.05$) higher at higher protein levels (40-50%) than all other diets. The crabs fed diet CP-40 showed the best percentage weight gain (wet wt. gain=1993.50%, dry wt. gain=2001.60%), followed by CP-45 (wet wt. gain=1803.60%, dry wt. gain=1814.20%). The weight gain was moderate when fed CP-50 (wet wt. gain=1615.00%, dry wt. gain=1638.50%), but substantially low in crabs fed CP-35 (wet wt. gain=634.37%, dry wt. gain=544.82%), and the lowest gain in weight was recorded in crabs fed CP-25 (wet wt. gain=82.04%, dry wt. gain=63.10%). The wet weight gain recorded with CP-40 was about 24 folds greater than that recorded with CP-25, and in the case of dry weight gain there was almost 31 folds increase.

Table-45: Amino acid composition of formulated diets containing graded protein levels (% of DM)

Amino acid	CP-15	CP-20	CP-25	CP-30	CP-35	CP-40	CP-45	CP-50
Arg	*0.86*	*1.01*	*1.30*	*1.62*	*1.78*	*2.10*	*2.35*	*2.64*
His	*0.40*	*0.48*	*0.62*	*0.77*	*0.84*	*0.99*	*1.11*	*1.20*
Ile	*0.43*	*0.50*	*0.65*	*0.81*	*0.89*	*1.05*	*1.17*	*1.27*
Leu	*1.21*	*1.42*	*1.84*	*2.29*	*2.51*	*2.96*	*3.31*	*3.58*
Lys	*1.94*	*2.28*	*2.95*	*3.78*	*4.10*	*4.76*	*5.31*	*5.75*
Met	*0.33*	*0.39*	*0.50*	*0.63*	*0.69*	*0.81*	*0.90*	*0.98*
Thr	*0.71*	*0.83*	*1.16*	*1.35*	*1.48*	*1.74*	*1.94*	*2.11*
Trp	*0.40*	*0.47*	*0.61*	*0.76*	*0.83*	*0.98*	*1.09*	*1.18*
Phe	*0.53*	*0.62*	*0.80*	*1.00*	*1.10*	*1.29*	*1.44*	*1.56*
Val	*0.58*	*0.69*	*0.89*	*1.11*	*1.22*	*1.44*	*1.60*	*1.74*
Ala	1.55	1.82	2.35	2.94	3.22	3.80	4.24	4.60
Asp	0.86	1.01	1.30	1.62	1.78	2.10	2.35	2.54
Cys	0.07	0.08	0.10	0.13	0.14	0.16	0.18	0.20
Glu	2.03	2.38	3.08	3.85	4.22	4.97	5.55	6.02
Gly	1.75	2.05	2.66	3.32	3.64	4.29	4.79	5.19
Pro	0.92	1.08	1.40	1.74	1.92	2.26	2.52	2.74
Ser	0.89	1.05	1.35	1.70	1.86	2.19	2.44	2.65
Tyr	0.41	0.49	0.63	0.78	0.86	1.01	1.13	1.23
Σ EAA	7.38	8.67	11.30	14.11	15.43	18.11	20.23	22.02
Σ NEAA	8.47	9.95	12.88	16.07	17.63	20.79	23.21	25.15
EAA/NEAA	0.87	0.87	0.88	0.88	0.88	0.87	0.87	0.88

- CP-15, CP-20, CP-25, CP-30, CP-35, CP-40, CP-45 and CP-50 are formulated feeds containing 15-50 % crude protein in graded levels.
- Values given in italics are essential amino acids.

4.4.3.2. Carapace width gain

The crabs had wider ($P<0.05$) carapaces, fed diet CP-40 (223.54%), followed by CP-45 and 50 (Table-46; Fig.18). Almost 2.19 folds increase in carapace width was seen in CP-40 compared to the lowest gain at CP-25 (CWG=102.04%). Only moderate gain in carapace width was observed in crabs fed CP-35 (151.68%) and CP-30 (128.25%). The carapace width gain in all the treatments was found to be significantly different ($P<0.05$) from each other.

4.4.3.3. Specific growth rate

The diets containing higher protein levels (40-50 %) induced significantly ($P<0.05$) greater specific growth rate (3.81-4.37%) in juvenile crabs (Table-46, Fig.19). The SGR recorded by crabs fed CP-40 and CP-50 were not significantly ($P>0.05$) different from CP-45. There were also no significant differences in the SGR between diets CP-30 and CP-35 (1.83-2.19%), but these were significantly different ($P<0.05$) from other treatments. The juvenile crabs fed diet CP-25, showed significantly ($P<0.05$) lower SGR (0.67%), than those fed other feeds.

4.4.3.4. Feed conversion ratio

Significant ($P<0.05$) improvement in feed efficiency was recorded in crabs fed CP-40, 45 and 50, with lower feed conversion ratio (1.86-1.98) and CP- 40 showed the lowest FCR among the three (Table-46, Fig-19). Significantly higher FCR was obtained with diet CP-25 containing only 25% of crude protein. Thus there was steady decrease in the efficiency of feed conversion with the increase in protein level in the diets.

4.4.3.5. Protein efficiency ratio

Significantly ($P<0.05$) higher PER (Table-46, Fig-19) resulted in juvenile crabs fed CP-40 (1.31), followed by CP-45 (1.14), CP-35 and CP-50 (0.96-1.01, $P>0.05$) and dietsCP-25 and CP-30 resulted in significantly ($P<0.05$) lower PER (0.67) than other diets.

4.4.3.6. Intermoult duration

The lower protein levels in CP-25, 30, and 35 resulted in significantly ($P<0.05$) longer intermoult duration in days, (Table-46, Fig- 20) in juvenile crabs fed those diets, where as crabs fed CP-40, CP-45 and CP-50 showed significantly (10.03-10.07 days/moult, $P<0.05$) shorter intermoult duration.

4.4.4. Proximate profile of the experimental crabs

The juvenile crabs fed the formulated diets with graded protein levels were also subjected to proximate analysis (Table-47). The dietary protein level had significant ($P<0.05$) influence on the proximate composition of the crabs.

4.4.4.1. Moisture and dry matter

The moisture and dry matter contents of the juvenile crabs (Table-47, Fig.21) fed the formulated diets containing graded levels of protein ranged from 71.70-90.55% and 9.45-28.30% respectively. The significantly ($P<0.05$) lower moisture content and higher dry matter contents (M=71.70-71.79%, DM=28.21-28.30%) were recorded in juvenile crabs fed CP-40, 45 and 50 followed by CP-35 (M=78.04%, DM=21.96%), and higher moisture hence lower dry matter were observed in crabs fed the diets CP-25 and 30 (M=84.29-89.74%, DM=10.26-15.71%). There were no significant ($P>0.05$) differences in moisture as well as dry matter contents of crabs among diets CP-40, 45 and 50, but were significantly ($P<0.05$) different from other diets containing lower levels of protein. Also significant ($P<0.05$) difference in moisture and dry matter contents was observed among CP-25, 30 and 35.

4.4.4.2. Crude protein

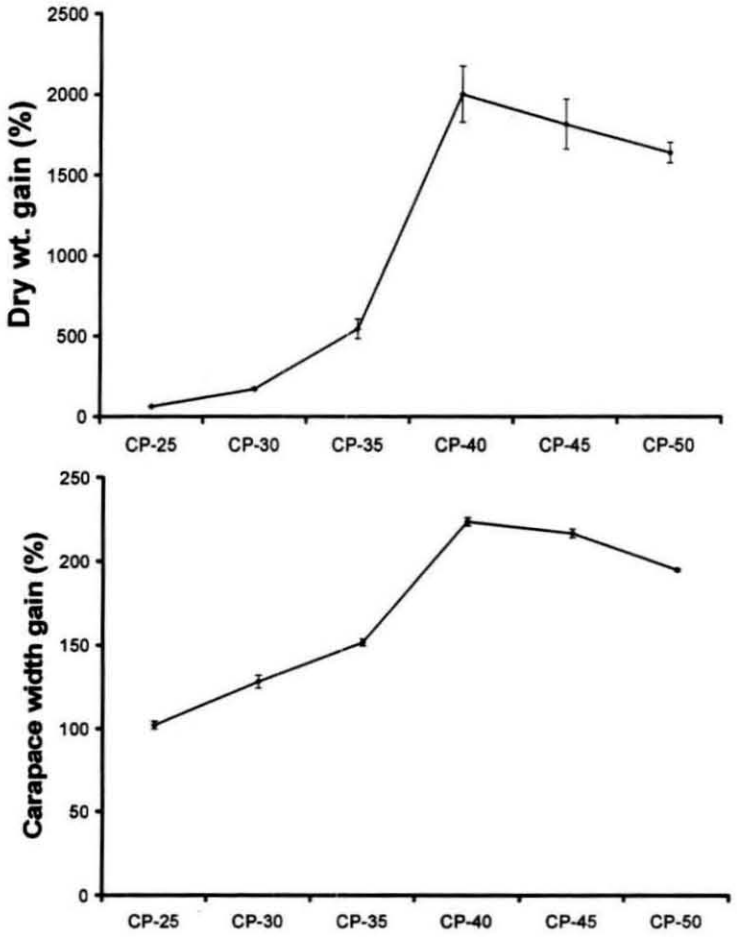
The carcass crude protein levels (Table-47, Fig.22) were significantly ($P<0.05$) affected by the protein levels in the diets. Significantly ($P<0.05$) higher crude protein content (11.05 %) was observed in the juvenile crabs fed the diet containing 50 % crude protein and significantly lower protein content (2.11%) in crabs fed CP-25 (25 % crude protein) among all the treatments. The crabs fed diets CP-45 (10.92%), CP-40 (10.73%) CP-35 (7.64%), also had relatively high crude

Table-46: Growth response of juvenile *Scylla serrata* fed formulated pellet diet containing graded protein levels

FEEDS	% SGR	FCR	PER	% WET WT. GAIN	% DRY WT. GAIN	% CW GAIN	MF (days/moult)
CP-25	0.67 ±0.11^{a}	5.99 ±0.27^{a}	0.67 ±0.03^{a}	82.04 ±9.93^{a}	63.10 ±3.44^{a}	102.04 ±2.35^{a}	17.67 ±0.30^{a}
CP-30	1.83 ±0.12^{b}	4.73 ±0.25^{b}	0.67 ±0.03^{a}	221.58 ±13.74^{b}	170.68 ±7.29^{a}	128.25 ±3.82^{b}	13.25 ±0.22^{b}
CP-35	2.19 ±0.28^{b}	2.98 ±0.23^{c}	0.96 ±0.08^{b}	634.37 ±82.03^{c}	544.82 ±60.86^{b}	151.68 ±2.00^{c}	10.63 ±0.15^{c}
CP-40	4.37 ±0.56^{c}	1.86 ±0.24^{d}	1.31 ±0.03^{c}	1993.50 ±182.26^{d}	2001.60 ±174.25^{c}	223.54 ±2.42^{d}	10.03 ±0.08^{d}
CP-45	4.20 ±0.48^{cd}	1.90 ±0.18^{d}	1.14 ±0.04^{d}	1803.60 ±161.43^{e}	1814.20 ±153.85^{d}	216.67 ±2.42^{e}	10.03 ±0.23^{d}
CP-50	3.81 ±0.24^{d}	1.98 ±0.28^{d}	1.01 ±0.10^{b}	1615.00 ±66.76^{f}	1638.50 ±63.78^{e}	194.93 ±0.96^{f}	10.07 ±0.10^{d}

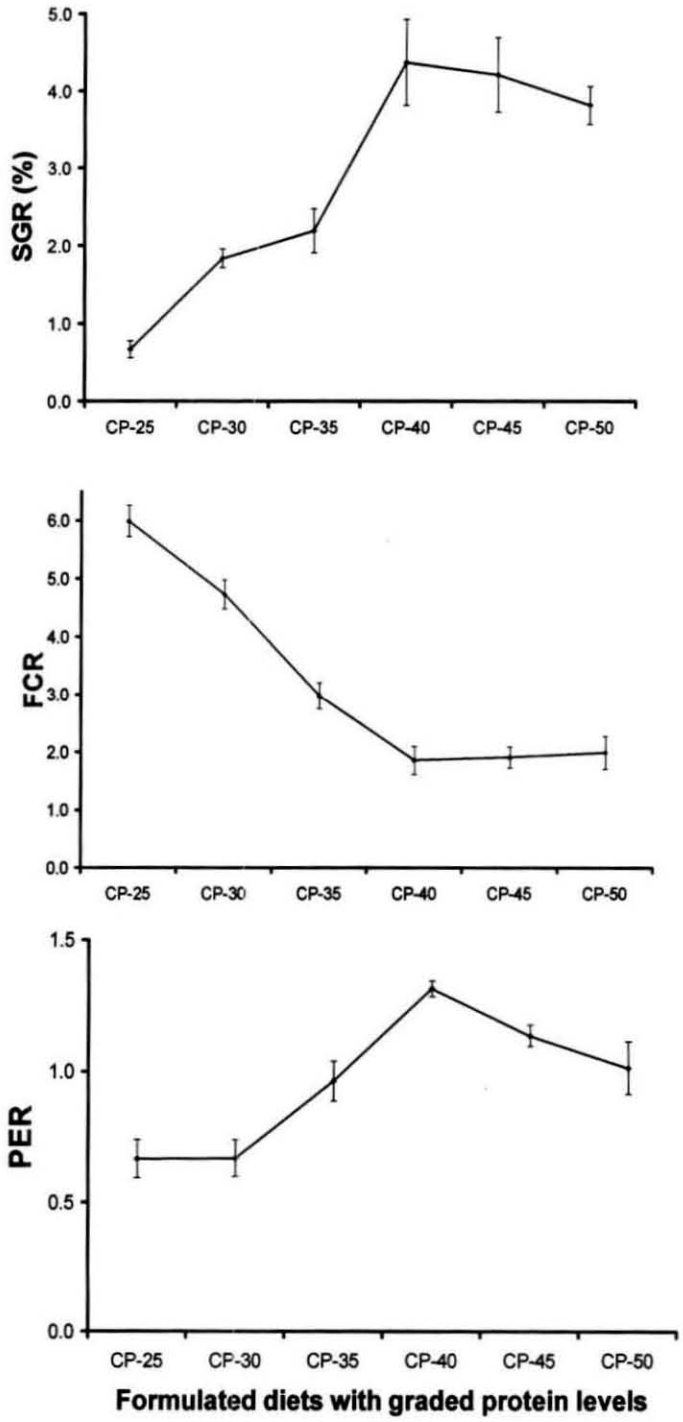
- CP-25, CP-30, CP-35, CP-40, CP-45 and CP-50 are formulated feeds containing 15-50 % crude protein in graded levels.
- Means in the columns with different superscript letters are significantly different ($P<0.05$).

Figure-18: Percentage moisture and dry matter contents of juvenile *Scylla serrata* fed with formulated diets having graded protein levels.



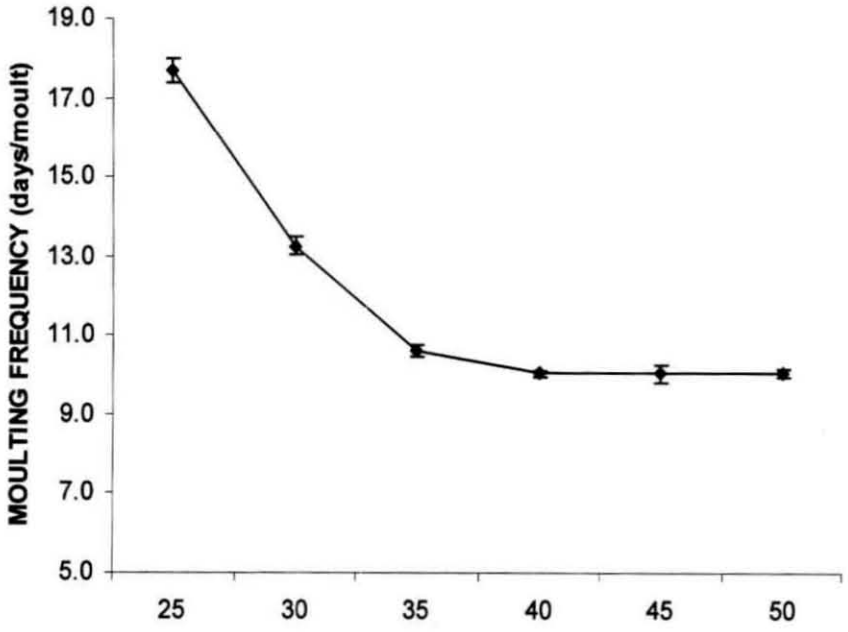
- CP-25, CP-30, CP-35, CP-40, CP-45 and CP-50 are formulated feeds containing 25-50 % crude protein in graded levels.

Figure-19: SGR, FCR and PER of juvenile *Scylla serrata* fed formulated feeda with graded protein level.



- CP-25, CP-30, CP-35, CP-40, CP-45 and CP-50 are formulated feeds containing 25-50 % crude protein in graded levels.

Fig-20: Moulting frequency of juvenile *Scylla serrata* fed formulated pellet diets containing graded protein levels



- CP-25, CP-30, CP-35, CP-40, CP-45 and CP-50 are formulated feeds containing 25-50 % crude protein in graded levels.

protein content as compared to the crabs fed diets CP-25 (2.11 %) and CP-30 (4.39%). There were no significant ($P>0.05$) differences between diets CP-40 and 45 and between CP-45 and 50 in the protein content of crabs. Similarly there were significant ($P<0.05$) differences in the protein content among the crabs fed diets CP-25, 30 and 35.

4.4.4.3. Crude lipid

The crude lipid level (Table-47, Fig.22) in the crabs varied from 0.17 % to 1.60 % and the crabs fed diets containing higher protein levels in the diets had significantly ($P<0.05$) higher crude lipid levels than those fed lower protein levels.

4.4.4.4. Crude fiber

The crude fiber content (Table-47, Fig-23), which reflects the chitin content in the crabs, was found to be significantly ($P<0.05$) higher in juvenile crabs fed CP-40, 45 and 50 (4.38-4.68%) followed by CP-35 (4.06%), CP-30 (3.45%) and relatively lower chitin level (2.68 %) was observed in crabs fed CP-25. No significant difference ($P>0.05$) in whole body crude fiber content was observed between CP-40 and 45 and also between CP-45 and 50, but these were significantly ($P<0.05$) different from all other treatments.

4.4.4.5. Crude ash

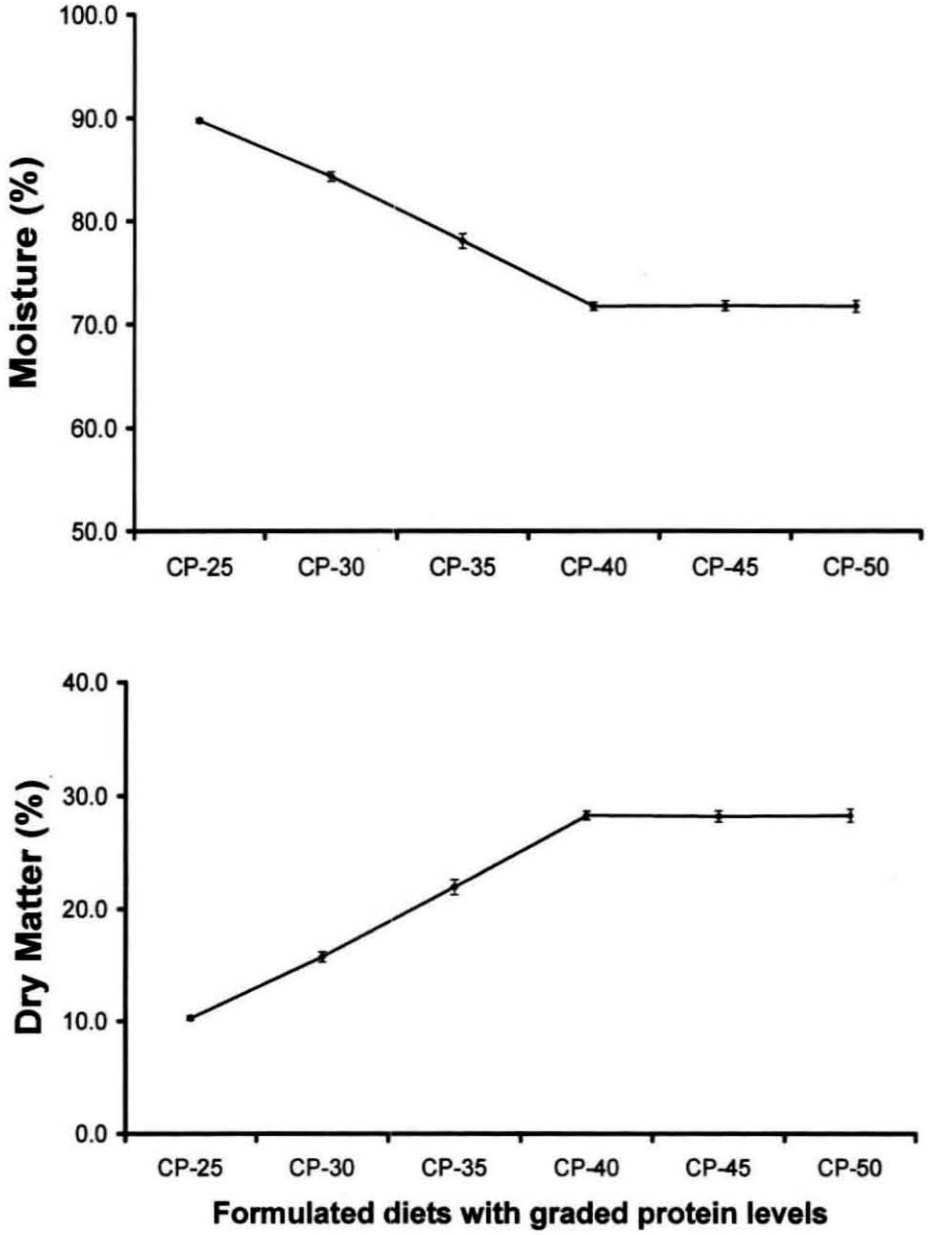
Among the juvenile crabs fed formulated diets containing graded protein levels significantly ($P<0.05$) greater mineral deposition (Table-47, Fig-23), in terms of crude ash was observed in crabs fed CP-40, 45 and 50 (9.64-9.76%) followed by CP-35 (7.94%, $P<0.05$), CP-30 (6.37%, $P<0.05$) and lower mineral deposition was observed in crabs fed CP-25 (4.59%, $P<0.05$). Thus there was a distinct direct relationship between the crude protein levels in the diets and the crude ash levels in the crabs.

Table-47: Proximate profile of the juvenile *Scylla serrata* fed formulated pellet diets containing graded protein levels (in % as such basis)

Feeds	Moisture	Dry matter	Crude protein	Crude lipid	Crude fiber	Crude ash	Nitrogen free extract	Gross energy (kJ/g)
CP-25	89.74 ±0.19 [a]	10.26 ±0.19 [a]	2.11 ±0.07 [a]	0.17 ±0.02 [a]	2.68 ±0.08 [a]	4.59 ±0.19 [a]	0.72 ±0.02 [a]	0.69 ±0.02 [a]
CP-30	84.29 ±0.45 [b]	15.71 ±0.45 [b]	4.39 ±0.23 [b]	0.65 ±0.11 [b]	3.45 ±0.23 [b]	6.37 ±0.46 [b]	0.86 ±0.11 [a]	1.44 ±0.08 [b]
CP-35	78.04 ±0.6 [c]	21.96 ±0.69 [c]	7.64 ±0.15 [c]	1.02 ±0.06 [c]	4.06 ±0.15 [c]	7.94 ±0.69 [c]	1.30 ±0.06 [b]	2.43 ±0.05 [c]
CP-40	71.70 ±0.38 [d]	28.30 ±0.38 [d]	10.73 ±0.16 [d]	1.53 ±0.12 [d]	4.68 ±0.16 [d]	9.76 ±0.38 [d]	1.60 ±0.12 [c]	3.42 ±0.07 [d]
CP-45	71.79 ±0.49 [d]	28.21 ±0.49 [d]	10.92 ±0.06 [de]	1.58 ±0.08 [d]	4.54 ±0.06 [de]	9.64 ±0.49 [d]	1.54 ±0.09 [c]	3.47 ±0.03 [d]
CP-50	71.72 ±0.57 [d]	28.28 ±0.57 [d]	11.05 ±0.17 [e]	1.60 ±0.06 [d]	4.38 ±0.17 [e]	9.74 ±0.57 [d]	1.51 ±0.06 [c]	3.51 ±0.03 [d]

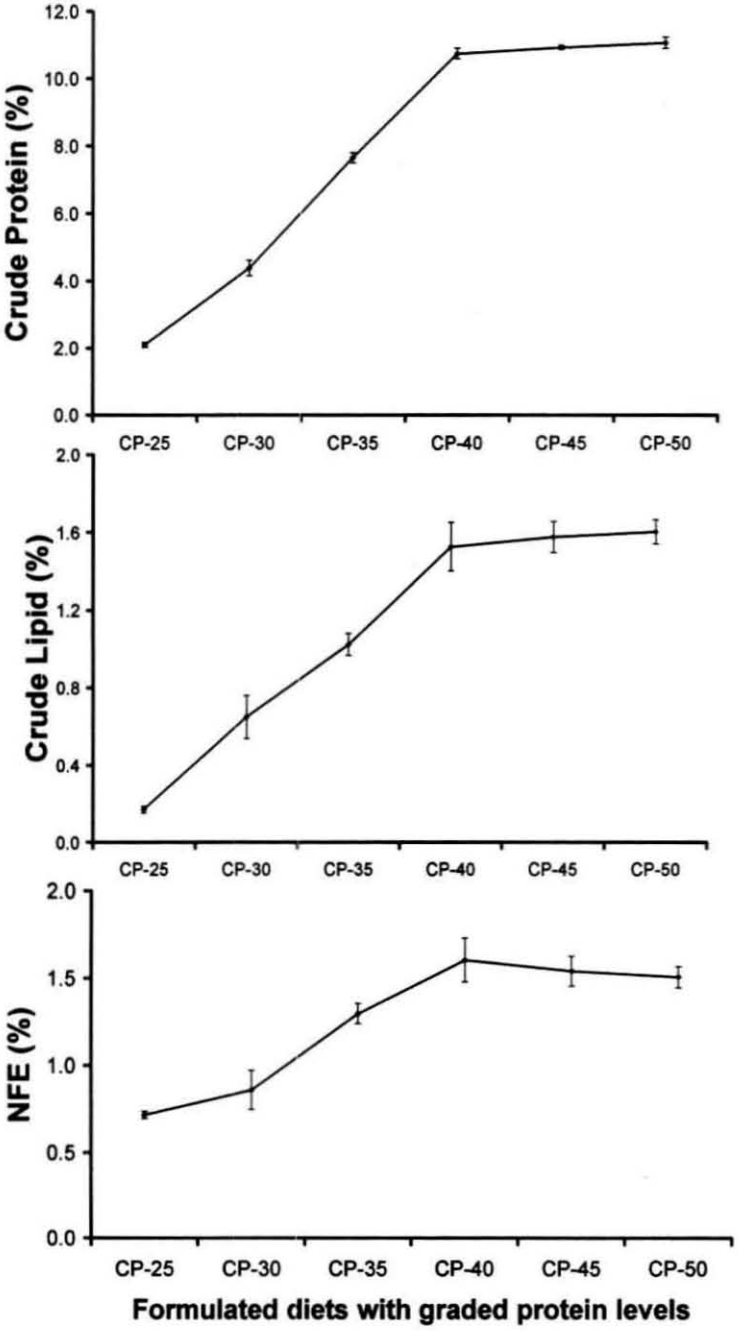
- CP-25, CP-30, CP-35, CP-40, CP-45 and CP-50 are formulated feeds containing 15-50 % crude protein in graded levels.
- Means in the columns with different superscript letters are significantly different ($P<0.05$).

Figure-21: Percentage moisture and dry matter contents of juvenile *Scylla serrata* fed with formulated diets having graded protein levels (as such basis).



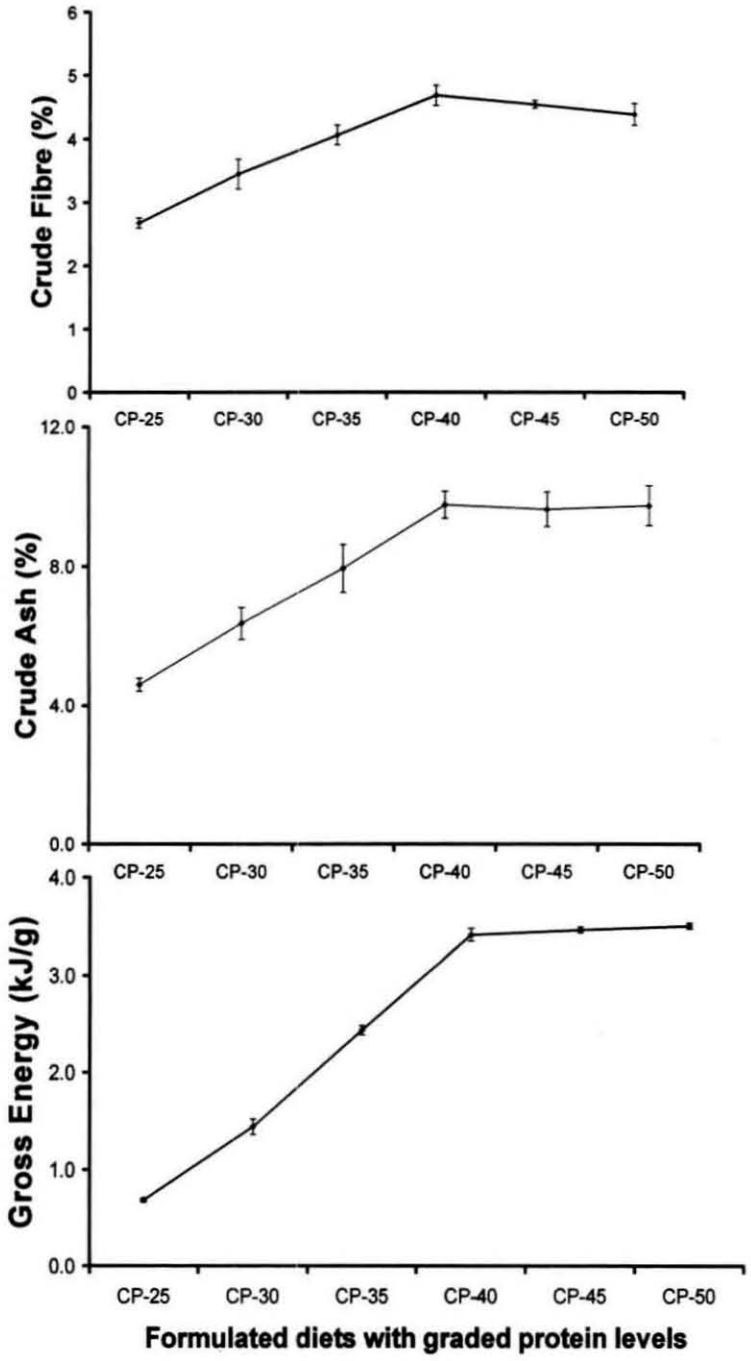
- CP-25, CP-30, CP-35, CP-40, CP-45 and CP-50 are formulated feeds containing 25-50 % crude protein in graded levels.
- NFE- Nitrogen free extract

Figure-22: Crude protein, crude lipid and nitrogen free extract (NFE) contents of juvenile *Scylla serrata* fed with formulated diets having graded protein levels (as such basis).



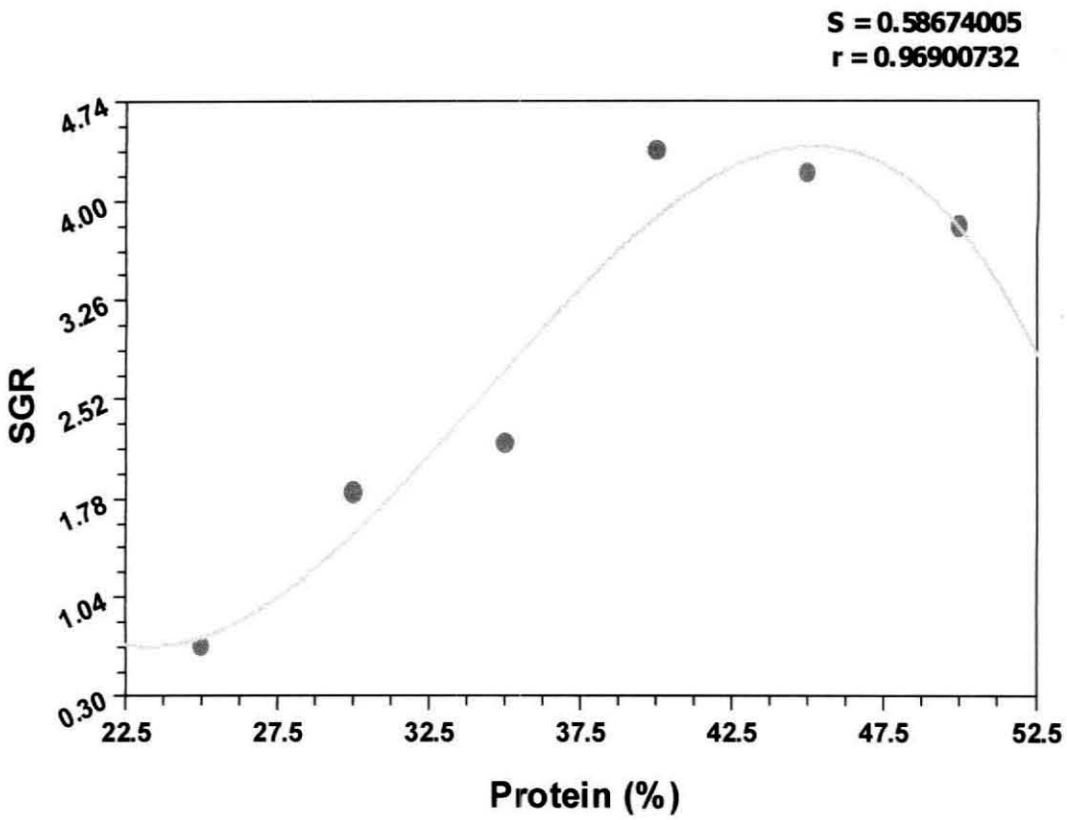
- CP-25, CP-30, CP-35, CP-40, CP-45 and CP-50 are formulated feeds containing 25-50 % crude protein in graded levels.
- NFE- Nitrogen free extract

Figure-23: Crude fibre, crude ash and gross energy contents of juvenile *Scylla serrata* fed with formulated diets having graded protein levels (as such basis).



- CP-25, CP-30, CP-35, CP-40, CP-45 and CP-50 are formulated feeds containing 25-50 % crude protein in graded levels.
- NFE- Nitrogen free extract

Figure-23A: Quadratic fit curve of SGR vs crude protein levels in feeds [y=a+bx+cx^2+dx^3..]



- Optimum crude protein level range 45.10% will give best SGR of 4.408

4.4.4.6. Nitrogen free extract

The nitrogen free extract levels (Table-47, Fig-22) of the crabs fed various diets ranged from 0.72 % to 1.60 %. The crabs fed CP-40, 45 and 50 (1.51-1.60%) had significantly ($P<0.05$) higher NFE levels than all other diets, but there were significant ($P>0.05$) difference among diets CP-40, CP-45 and CP-50, and also among CP-25, 30 and 35 (0.72-1.30 %).

4.4.4.7. Gross energy

As in the case of the proximate principles, significantly ($P<0.05$) higher gross energy content (Table-47, Fig-23) was found in juvenile crabs fed higher protein level diets, CP-40, 45 and 50 (3.42-3.51 kJ/g) than those fed lower protein level diets CP-25, CP-30 (0.69-1.44 kJ/g), where as moderate energy content was observed in crabs fed CP-35 (2.43 kJ/g).

4.4.5. Apparent digestibility

Apparent digestibility of the diets (Table-48) was found significantly ($P<0.05$) influenced by the dietary protein level with a progressive increase in digestibility with the increase in protein levels in the diets. Significantly ($P<0.05$) higher apparent digestibility was recorded for feeds CP-40, 45 and 50 which ranged between 88.97 and 89.79%, followed by CP-35 (79.66%) and CP-30 (73.25%) where as CP-25 produced the lowest (62.25%) ADC.

4.5. Evaluation of lipid sources in formulated diets

4.5.1. Fatty acids profile of the diets

The fatty acids profile of the diets (Table-49) showed that the levels of saturated fatty acids ranged from 1.91-2.42 % with diet T-1 containing cod liver oil supplement having the highest level of saturated fatty acids (2.59 %). The unsaturated fatty acids in all the diets ranged from 8.02 to 8.68 %. The lowest level of mono-unsaturated fatty acids was found in diet T-3 (2.71 %) supplemented with soybean oil, where as all other diets had monosaturates in the range 3.43 to 4.72 %. Diets T-2 and T-3 showed a superior PUFA content (4.83-5.13 %), followed by

Table-48: Apparent digestibility coefficient (ADC) of formulated pellet diets containing graded protein levels for juvenile *Scylla serrata*

FEEDS	ADC	FCR
CP-25	62.25 ±1.79[a]	5.99 ±0.27[a]
CP-30	73.25 ±1.40[b]	4.73 ±0.25[b]
CP-35	79.66 ±7.05[c]	2.98 ±0.23[c]
CP-40	89.79 ±2.33[d]	1.86 ±0.24[d]
CP-45	89.37 ±3.13[d]	1.90 ±0.18[d]
CP-50	88.97 ±2.55[d]	1.98 ±0.28[d]

- CP-25, CP-30, CP-35, CP-40, CP-45 and CP-50 are formulated feeds containing 15-50 % crude protein in graded levels.
- Means in the columns with different superscript letters are significantly ($P<0.05$) different.

mixed oil supplements, and the lowest PUFA content was recorded in diet T-1 (cod liver oil supplemented). The n6 fatty acids were found to dominate in diets T-2 and T-3 (4.36-4.63 %), followed by diets T-4, T-5 and T-6 (with mixed oil supplements). The lowest n6 content (1.23 %) was recorded in diet T-1 supplemented with cod liver oil. Total n3 fatty acids were found to be more in diet T-1 followed by mixed oil supplements (1.51-1.64 %) where as diets T-2 and T-3 had very low levels (0.76-1.25 %) of n3. Diet T-1 had the highest n3 HUFA content (1.81 %) where as the lowest n3 HUFA content was recorded in diets T-2 and T-3. n3 HUFA content in diets containing mixed oil supplements were moderate and ranged from 1.10 to 1.11 %. The ratio of saturated to unsaturated fatty acids in the diets ranged from 0.22 to 0.32. The highest n3/n6 ratio was observed in diet T-1 (1.76), where as in diets supplemented with the oil mixtures it ranged from 0.52 to 0.58.

Essential fatty acids

Linoleic acid (LA, 18:2n6): Diets T-2 and T-3 had the highest 18:2n6 levels (4.21-4.40 %), where as in diets T-4, T-5 and T-6 the levels fluctuated between 2.62 and 2.68 %. Diet T-1 supplemented with cod liver oil had the lowest 18:2n6 levels (0.97 %).

Linolenic acid (LNA, 18:3n3): The diets supplemented with soybean oil (T-3) had the highest 18:3n3 content (0.82 %), followed by diets supplemented with mixtures of oil (T-4, T-5 and T-6; 0.40-0.54 %) and soybean oil (T-2; 0.34 %).

Arachidonic acid (AA, 20:4n6): The arachidonic acid levels in the various diets ranged from 0.10 to 0.11 %.

Eicosapentaenoic acid (EPA, 20:5n3): Diet supplemented with cod liver oil (T-1) had the highest EPA content (0.63 %) followed by diets T-4, T-5 and T-6 (0.41 %) and diets T-2 and T-3 had the lowest EPA content (0.20 %).

Docosahexaenoic acid (DHA, 22:6n3): The highest DHA content (1.18 %) was recorded in diet T-1 (cod liver oil supplemented) and diets T-2 and T-3 had the lowest level (0.22 %) of DHA. Diets supplemented with oil mixtures had moderate levels of DHA (0.69-0.70 %).

Table-49: Fatty acids profile of formulated pellet diets supplemented with various lipid sources (% of DM)

Fatty acid	T-1	T-2	T-3	T-4	T-5	T-6
C12:0	0.02	0.00	0.00	0.01	0.01	0.01
C14:0	0.53	0.15	0.18	0.35	0.35	0.35
C14:1n7	0.02	0.01	0.01	0.01	0.01	0.01
C15:0	0.04	0.02	0.02	0.03	0.03	0.03
C16:0	1.39	1.30	1.48	1.39	1.36	1.41
C16:1n7	1.11	0.25	0.25	0.67	0.67	0.67
C16:2n6	0.06	0.00	0.00	0.03	0.03	0.03
C17:0	0.12	0.07	0.07	0.10	0.09	0.09
C17:1n6	0.10	0.14	0.06	0.10	0.11	0.09
C18:0	0.49	0.37	0.61	0.49	0.45	0.53
C18:1n9	2.20	2.64	2.05	2.28	2.36	2.19
C18:1n7	0.27	0.16	0.11	0.20	0.21	0.20
C18:2n6	0.97	4.40	4.21	2.65	2.68	2.62
C18:3n3	0.36	0.34	0.82	0.47	0.40	0.54
C18:4n3	0.00	0.00	0.01	0.00	0.00	0.00
C20:1n11	0.87	0.08	0.08	0.47	0.48	0.47
C20:4n6	0.10	0.10	0.10	0.10	0.10	0.11
C20:5n3	0.63	0.20	0.20	0.41	0.41	0.41
C22:1n11	0.15	0.15	0.15	0.15	0.15	0.15
C22:6n3	1.18	0.22	0.22	0.70	0.70	0.69
C24:1n3	0.00	0.00	0.00	0.00	0.00	0.00
Σ Saturated	2.59	1.91	2.36	2.37	2.29	2.42
Σ unsaturated	8.02	8.68	8.25	8.24	8.31	8.18
Σ MUFA	4.72	3.43	2.71	3.88	3.99	3.78
Σ PUFA	1.43	4.83	5.13	3.22	3.18	3.27
Σ n3	2.17	0.76	1.25	1.58	1.51	1.64
Σ n6	1.23	4.63	4.36	2.88	2.92	2.85
Σ n3 HUFA	1.81	0.42	0.42	1.11	1.11	1.10
Saturated : Unsaturated	0.32	0.22	0.29	0.29	0.28	0.30
n3 : n6	1.76	0.16	0.29	0.55	0.52	0.58
DHA : EPA	1.87	1.10	1.10	1.71	1.71	1.68

- T-1, T-2, and T-3 are single oil supplements of cod liver oil, sunflower oil and soybean oil respectively.
- T4, T-5 and T-6 are oil mixtures containing cod liver oil, sunflower oil and soybean oil at ratios of 5:2.5:2.5, 5:4:1 and 5:1:4 respectively.

DHA/ EPA ratio: DHA/EPA ratio of diet T-1 was found to be the highest (1.87) and the ratios obtained with diets supplemented with mixed oil supplements ranged from 1.68 to 1.71. The lowest ratio (1.10) was recorded in diets supplemented with sunflower oil (T-2) and soybean oil (T-3).

4.5.2. Survival

Survival was not affected by the oil supplements in the diets and cent percent survival was obtained in all the treatments.

4.5.3. Growth response

4.5.3.1. Weight gain

No significant ($P>0.05$) difference was observed (Table-50, Fig.25) in the percentage wet and dry weight gains (wet wt. gain=1466.08-1471.35%; dry wt. gain=1435.40-1454.31%) among crabs fed the diets. However the weight gains attained by crabs fed diets T-1, T-4, T-5 and T-6 were significantly higher ($P<0.05$) than that obtained with crabs fed diets T-2 and T-3 (wet wt. gain=442.69-672.51%, $P<0.05$; dry wt. gain=155.58-208.22%, $P>0.05$).

4.5.3.2. Carapace width gain

Significantly higher ($P<0.05$) carapace width gain (Table-50, Fig.25) of crabs was obtained by feeding diets T-1, T-4, T-5 and T-6 (110.12-111.13%, $P>0.05$) than by feeding diets T-2 and T-3 (93.25% and 102.13%, $P>0.05$).

4.5.3.3. Specific growth rate

Significantly ($P>0.05$) higher SGR (Table-50, Fig-26) was recorded in crabs fed diet T-1 (3.95%) which was not significantly ($P>0.05$) different from the SGRs recorded for diets T-4, T-5 and T-6. Diets T-2 and T-3 showed significantly ($P>0.05$) lower SGRs (2.49-2.55%) than crabs fed other diets.

4.5.3.4. Feed conversion ratio

Significantly (P<0.05) lower FCR (Table-50, Fig-26) indicative of better performance was recorded in crabs fed with diets T-1, T-4, T-5 and T-6 (1.19-1.22, P>0.05) where as diets T-2 and T-3 recorded significantly higher FCR (1.33-1.35, P>0.05), suggesting poor food conversion of the ingested food.

4.5.3.5. Protein efficiency ratio

PER (Table-50, Fig-26) also showed a similar trend as that of SGR, and the crabs fed diets T-1, T-4, T-5 and T-6 gave the best PER (1.98-2.02, P>0.05) and the PER obtained for diets T-2 and T-3 (1.75-1.80, P>0.05) were significantly (P<0.05) lower than the other feeds.

4.5.3.6. Intermoult duration

Diets T-1, T-4, T-5 and T-6 (Table-50, Fig-27) resulted in significantly (P<0.05) shorter intermoult intervals (10.30-10.33 days/moult, P>0.05) than those fed with T-2 and T-3 (13.71 and 13.38 days/moult P<0.05).

4.5.4. The proximate profile of the experimental crabs

The results of the proximate analysis (as such basis) of the experimental crabs are presented in Table-51 and Fig. 28 to 30.

4.5.4.1. Moisture & Dry matter

Juvenile crabs fed diets T-1, T-4, T-5 and T-6 had significantly (P<0.05) lower moisture and higher dry matter contents (M=70.71-70.98%, DM=29.02-29.29%, P>0.05) than those fed diet T-2 (M=88.19%, DM=11.81%; P<0.05) and diet T-3 (M=86.06%, DM=13.94%; P<0.05) (Table-51, Fig- 28).

4.5.4.2. Crude protein

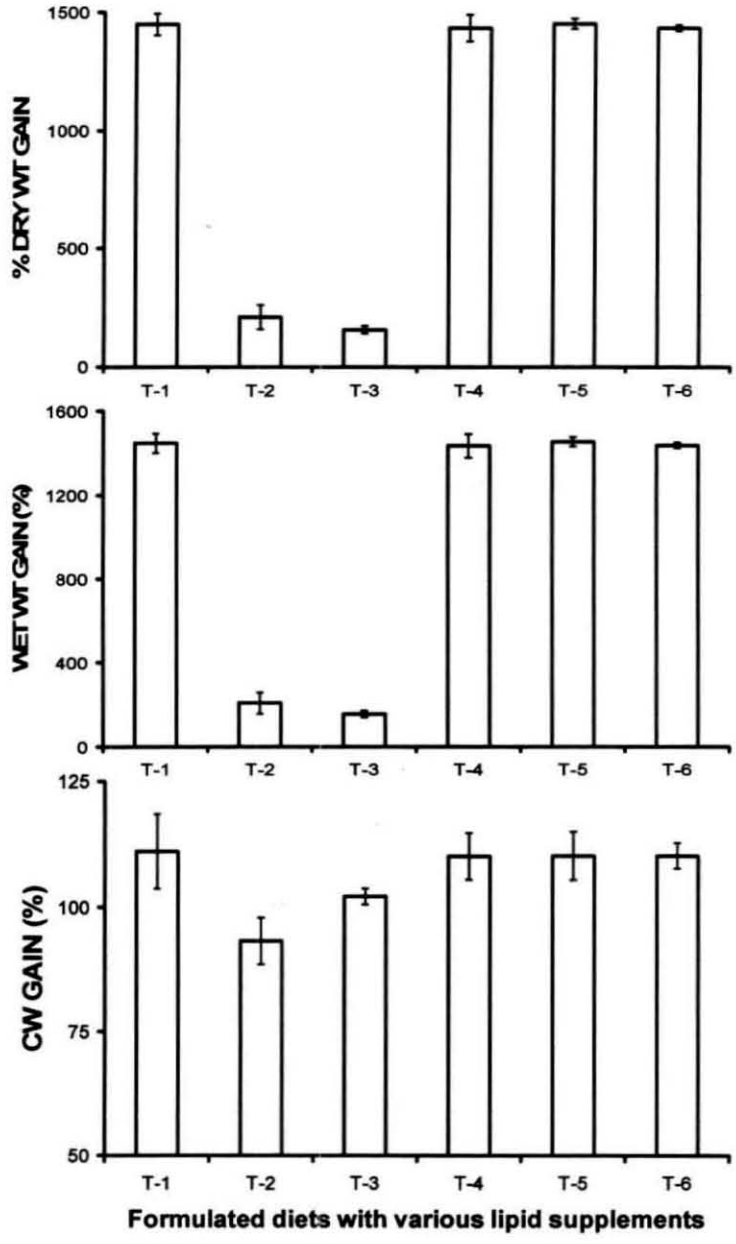
Significantly (P<0.05) higher crude protein accretion (Table-51, Fig- 29) was observed in the crabs fed diets T-1 and T-5 (11.66-11.83%, P>0.05) followed by T-4 and T-6 (11.38-11.45%, P>0.05), and significantly (P<0.05) lower crude protein content was found in crabs fed T-3 (4.19%) and T-2 (3.52%).

Table-50: Growth response of juvenile *Scylla serrata* fed formulated diets incorporated with cod liver oil, sunflower oil and soybean oil, individually and in combinations

FEEDS	% SGR	FCR	PER	% WET WT. GAIN	% DRY WT. GAIN	% CW GAIN	MF (days/moult)
T-1	3.95 ±0.09[a]	1.20 ±0.01[a]	1.99 ±0.07[a]	1471.35 ± 46.00[a]	1449.05 ±45.35[a]	111.13 ±7.40[a]	10.30 ±0.21[a]
T-2	2.49 ±0.25[b]	1.35 ±0.05[b]	1.75 ±0.03[b]	672.51 ±50.48[b]	208.22 ±50.61[b]	93.28 ±4.65[b]	13.71 ±0.29[b]
T-3	2.55 ±0.03[b]	1.33 ±0.03[b]	1.80 ±0.02[b]	442.69 ±15.78[c]	155.58 ±15.61[b]	102.13 ±1.50[c]	13.38 ±0.26[c]
T-4	3.85 ±0.10[a]	1.22 ±0.04[a]	1.99 ±0.05[a]	1466.08 ±56.60[a]	1435.40 ±55.49[a]	110.12 ±4.62[a]	10.33 ±0.16[a]
T-5	3.95 ±0.04[a]	1.22±0.02[a]	1.98 ±0.02[a]	1470.76 ±21.90[a]	1454.31 ±21.67[a]	110.20 ±4.80[a]	10.33 ±0.16[a]
T-6	3.98 ±0.10[a]	1.19 ±0.02[a]	2.02 ±0.07[a]	1466.67 ±13.13[a]	1437.03 ±12.88[a]	110.21 ±2.55[a]	10.33 ±0.24[a]

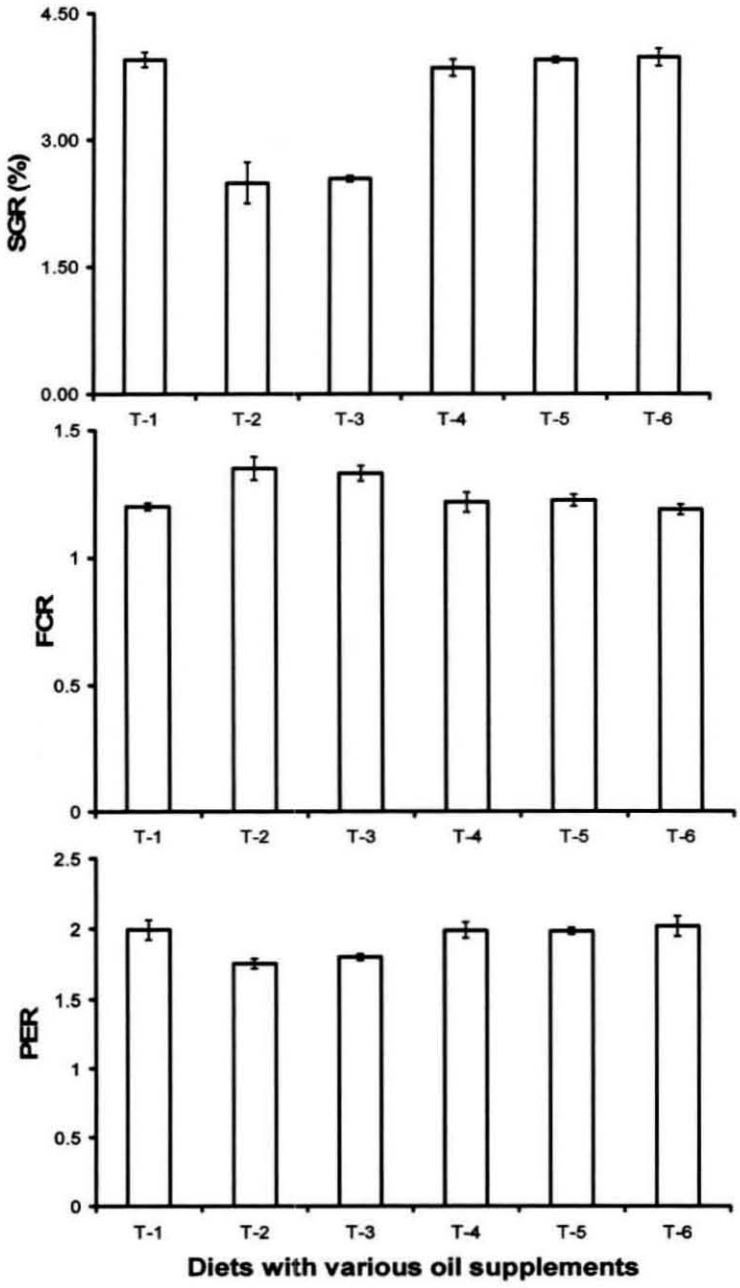
- T-1, T-2, and T-3 are single oil supplements of cod liver oil, sunflower oil and soybean oil respectively.
- T4, T-5 and T-6 are oil mixtures containing cod liver oil, sunflower oil and soybean oil at ratios of 2:1:1, 5:4:1 and 5:1:4 respectively.
- Means in columns with different superscript letters are significantly different ($P<0.05$).

Figures-24: Percentage wet weight, dry weight and carapace width gain of juvenile *Scylla serrata* fed formulated diets supplemented with various lipid sources



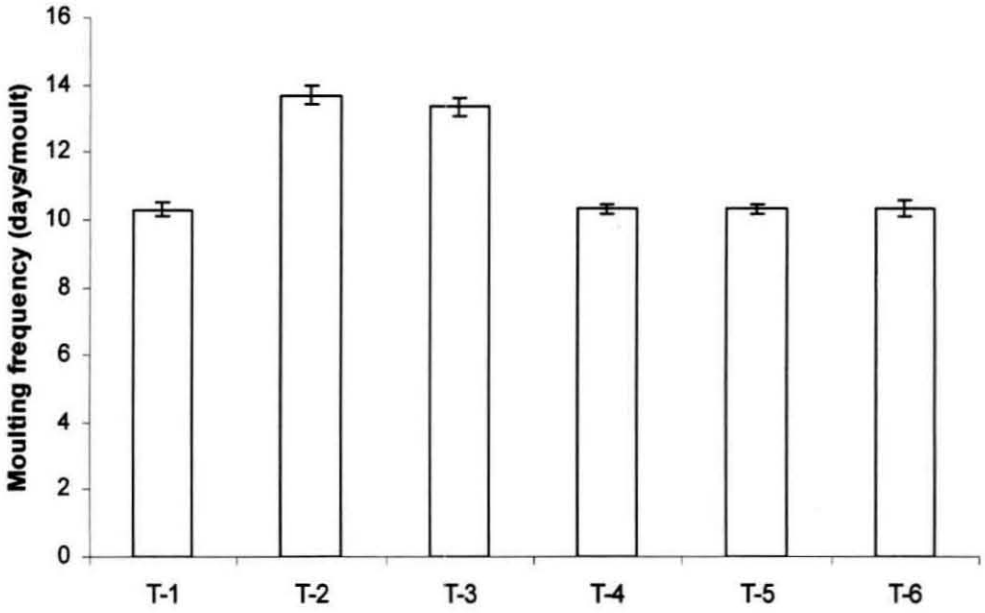
- T-1, T-2, and T-3 are single oil supplements of cod liver oil, sunflower oil and soybean oil respectively.
- T4, T-5 and T-6 are oil mixtures containing cod liver oil, sunflower oil and soybean oil at ratios of 5:2.5:2.5, 5:4:1 and 5:1:4 respectively.

Figures-25: SGR, FCR and PER of juvenile *Scylla serrata* fed formulated diets supplemented with various lipid sources



- T-1, T-2, and T-3 are single oil supplements of cod liver oil, sunflower oil and soybean oil respectively.
- T4, T-5 and T-6 are oil mixtures containing cod liver oil, sunflower oil and soybean oil at ratios of 5:2.5:2.5, 5:4:1 and 5:1:4 respectively.

Fig-26: Moulting frequency of juvenile *Scylla serrata* fed formulated pellet diets supplemented with various lipid sources



- T-1, T-2, and T-3 are single oil supplements of cod liver oil, sunflower oil and soybean oil respectively.
- T4, T-5 and T-6 are oil mixtures containing cod liver oil, sunflower oil and soybean oil at ratios of 5:2.5:2.5, 5:4:1 and 5:1:4 respectively.

4.5.4.3. Crude lipid

Significantly ($P<0.05$) higher crude lipid levels (Table-51, Fig- 29) were found in juvenile crabs fed diet T-1 (1.67%), followed by T-4, T-5 and T-6 (1.60-1.62%, $P<0.05$) and significantly ($P<0.05$) lower crude lipid level was found in crabs fed diets T-2 and T-3 (0.74-0.79%, $P>0.05$).

4.5.4.4. Crude fiber

Crude fiber content, which reflects the chitin content of the shell, was significantly ($P<0.05$) higher in crabs fed T-1, T-4, T-5 and T-6 (4.64-4.87%, $P>0.05$) than juvenile crabs fed T-2 and T-3 (2.73, 3.33; $P<0.05$) (Table-51, Fig- 30).

4.5.4.5. Crude ash

Crude ash was significantly ($P<0.05$) higher in juvenile crabs fed formulated diets T-1, T-4, T-5 and T-6 (9.79-10.05%, $P>0.05$), whereas, significantly lower ($P<0.05$) ash level was found in crabs fed T-2 and T-3 (4.23-4.89%, $P>0.05$) (Table-51, Fig- 30).

4.5.4.6. Nitrogen free extract (NFE)

Juvenile crabs fed diets; T-1, T-4, T-5 and T-6 had NFE in significantly ($P<0.05$) greater levels (1.25-1.32%, $P>0.05$) than those crabs fed T-2 and T-3 (0.58-0.74%, $P>0.05$) (Table-51, Fig- 29).

4.5.4.7. Gross energy

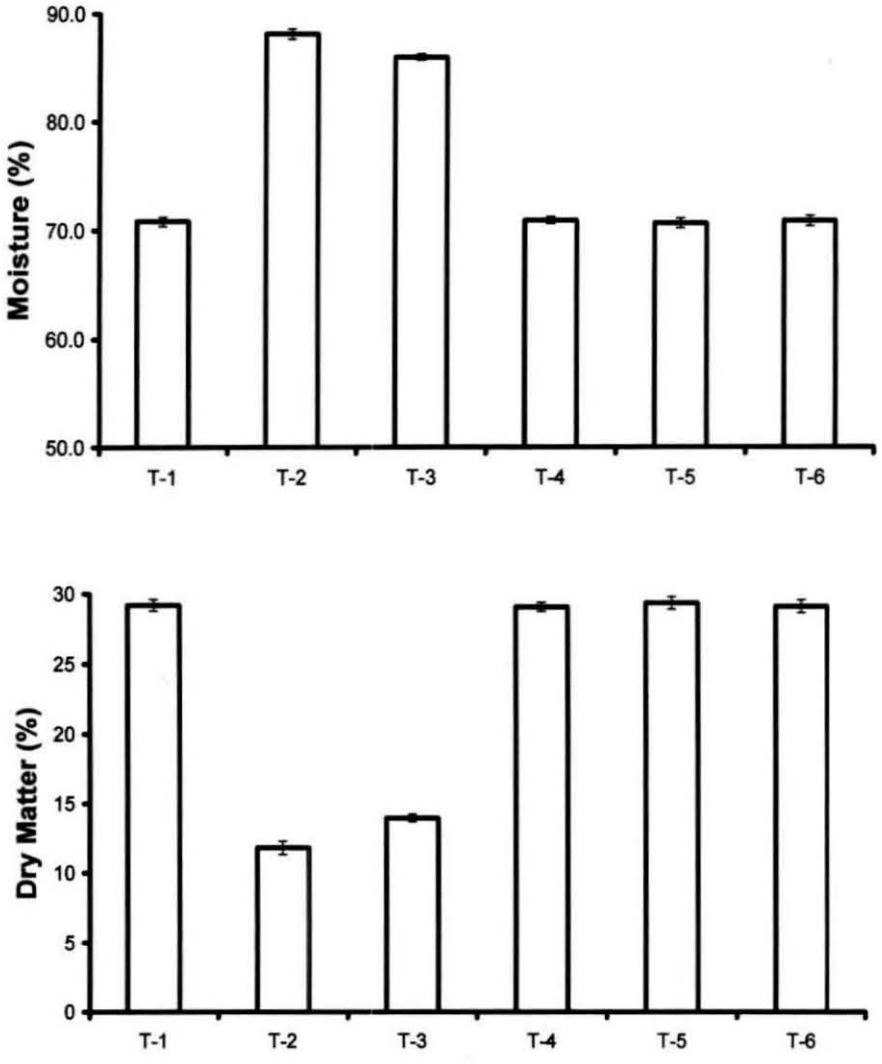
The gross energy content (Table-51, Fig- 30) of crabs fed formulated diets with various lipid supplements ranged from 1.22-3.67 kJ/g wet crab sample. Significantly ($P<0.05$) higher gross energy content was observed in juvenile crabs fed T-1 and T-5 (3.62-3.67 kJ/g, $P>0.05$) followed by T-4 and T-6 (3.55-3.56 kJ/g $P>0.05$). The crabs fed diets T-2 and T-3 had energy levels1.22 kJ/g and1.43 kJ/g respectively, which were significantly ($P<0.05$) lower compared to other diets.

Table-51: Proximate profile of the juvenile *Scylla serrata* fed formulated pellet diets supplemented with various lipid sources

Lipid source	Moisture	Dry matter	Crude protein	Crude lipid	Crude fiber	Crude ash	Nitrogen free extract	Gross energy (kJ/g)
T-1	70.82 ±0.42^{a}	29.18 ±0.42^{a}	11.83 ±0.10^{a}	1.67 ±0.19^{a}	4.64 ±0.11^{a}	9.79 ±0.94^{a}	1.25 ±0.09^{a}	2.82 ±0.13^{a}
T-2	88.19 ±0.48^{b}	11.81 ±0.48^{b}	3.52 ±0.14^{b}	0.74 ±0.09^{b}	2.73 ±0.07^{b}	4.23 ±0.58^{b}	0.58 ±0.12^{b}	0.95 ±0.12^{b}
T-3	86.06 ±0.27^{c}	13.94 ±0.27^{c}	4.19 ±0.33^{c}	0.79 ±0.10^{b}	3.33 ±0.21^{c}	4.89 ±0.35^{b}	0.74 ±0.08^{b}	1.10 ±0.17^{c}
T-4	70.98 ±0.33^{a}	29.02 ±0.33^{a}	11.45 ±0.65^{de}	1.61 ±0.11^{c}	4.77 ±0.15^{a}	9.91 ±0.53^{a}	1.29 ±0.09^{a}	2.74 ±0.28^{de}
T-5	70.71 ±0.45^{a}	29.29 ±0.45^{a}	11.66 ±0.31^{ae}	1.62 ±0.13^{c}	4.64 ±0.18^{a}	10.05 ±0.34^{a}	1.32 ±0.10^{a}	2.78 ±0.18^{ae}
T-6	70.96 ±0.48^{a}	29.04 ±0.48^{a}	11.38 ±0.37^{d}	1.60 ±0.14^{c}	4.87 ±0.11^{a}	9.88 ±0.29^{a}	1.31 ±0.05^{a}	2.72 ±0.19^{d}

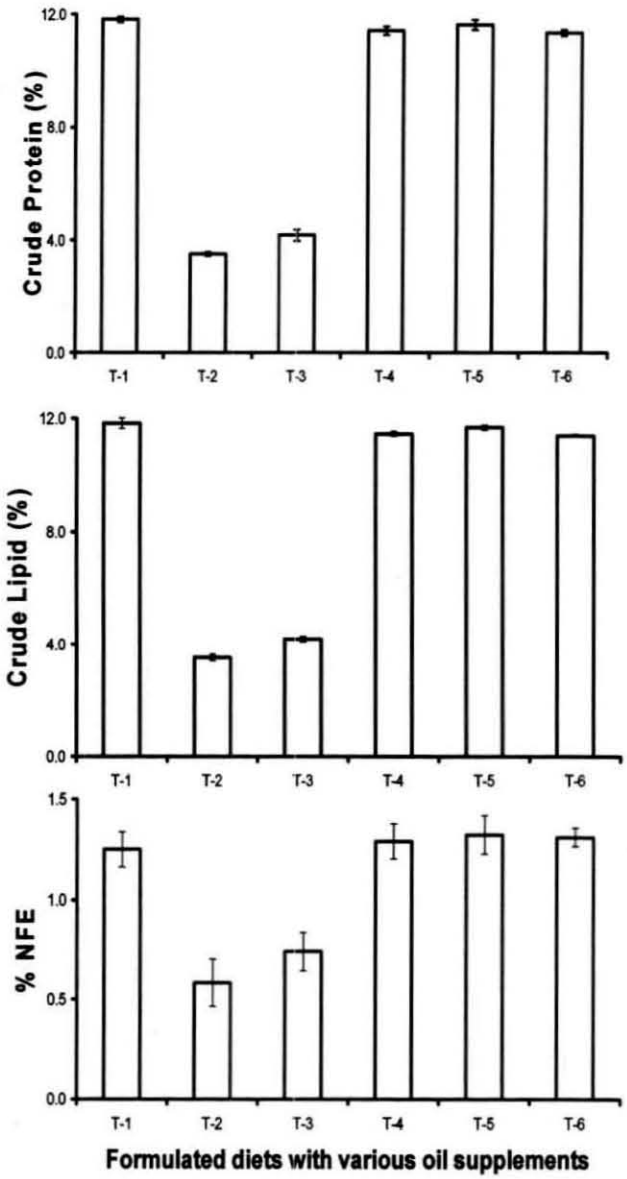
- T-1, T-2, and T-3 are single oil supplements of cod liver oil, sunflower oil and soybean oil respectively.
- T4, T-5 and T-6 are oil mixtures containing cod liver oil, sunflower oil and soybean oil at ratios of 2:1:1, 5:4:1 and 5:1:4 respectively.
- Means with different superscript letters are significantly different ($P<0.05$).

Figure-27: Percentage moisture and dry matter contents of juvenile *Scylla serrata* fed formulated pellet diet supplemented with various lipid supplements.



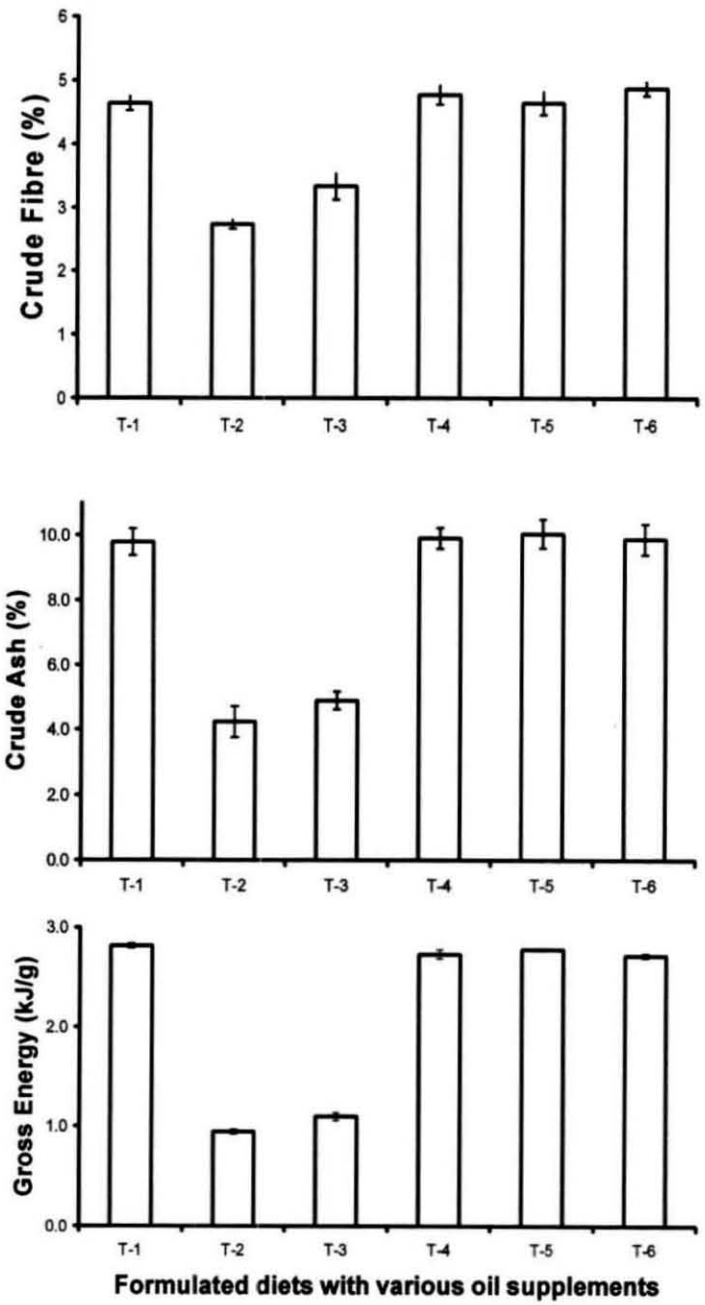
- T-1, T-2, and T-3 are single oil supplements of cod liver oil, sunflower oil and soybean oil respectively.
- T4, T-5 and T-6 are oil mixtures containing cod liver oil, sunflower oil and soybean oil at ratios of 5:2.5:2.5, 5:4:1 and 5:1:4 respectively.

Figure-28: Crude protein, crude lipid and nitrogen free extract (NFE) contents of juvenile *Scylla serrata* fed fresh and processed feeds (as such basis).



- T-1, T-2, and T-3 are single oil supplements of cod liver oil, sunflower oil and soybean oil respectively.
- T4, T-5 and T-6 are oil mixtures containing cod liver oil, sunflower oil and soybean oil at ratios of 5:2.5:2.5, 5:4:1 and 5:1:4 respectively.
- NFE – Nitrogen free extract

Figure-29: Crude fibre, crude ash and gross energy contents of juvenile *Scylla serrata* fed fresh and processed feeds (as such basis).



- T-1, T-2, and T-3 are single oil supplements of cod liver oil, sunflower oil and soybean oil respectively.
- T4, T-5 and T-6 are oil mixtures containing cod liver oil, sunflower oil and soybean oil at ratios of 5:2.5:2.5, 5:4:1 and 5:1:4 respectively.
- GE - Gross energy

4.5.5. Apparent digestibility

The formulated diets (Table-52) T-1, T-4, T-5 and T-6 fed to the juvenile *Scylla serrata* showed significantly ($P<0.05$) higher apparent digestibility (89.77-90.25 %) than diets T-2 and T-3 (85.63-86.47 %).

4.5.6. Multiple linear regression (MLR) analysis

The growth responses such as SGR (Table-53), FCR (Table-54) and PER (Table-55) were subjected to MLR analysis against the fatty acids profile of the formulated feeds containing various lipid sources. Among the saturated fatty acids 14:0 was found to influence the SGR ($R^2 = 0.751$), FCR ($R^2 = 0.773$) and PER ($R^2 = 0.713$) whereas among the mono-unsaturates 20:1n11 was found to influence SGR ($R^2 = 0.741$), FCR ($R^2 = 0.754$) and PER ($R^2 = 0.688$). Among the PUFAs the SGR was influenced by 18:2n6 ($R^2 = 0.739$), where as FCR ($R^2 = 0.877$), and PER ($R^2 =0.821$), were influenced by 18:2n6 and 20:4n6. SGR was further influenced by n3 fatty acids like 22:6n3 and 20:5n3 ($R^2 = 0.860$) where as FCR ($R^2 = 0.751$) and PER ($R^2 = 0.686$) were influenced by 22:6n3 alone. A similar trend was shown by n3 HUFAs also (SGR: predictor(s)-22:6n3 and 20:5n3, $R^2 = 0.860$; FCR: predictor(s) - 22:6n3, $R^2 = 0.751$; PER: predictor(s)-22:6n3, $R^2 = 0.686$).

Table-52: Apparent digestibility coefficients of formulated pellet diets containing various oil supplements when fed to juvenile *Scylla serrata*

FEEDS	ADC (%)	FCR
T-1	90.05 ±1.25[a]	1.20 ±0.01[a]
T-2	85.63 ±1.01[b]	1.35 ±0.05[b]
T-3	86.47 ±1.14[b]	1.33 ±0.03[b]
T-4	89.99 ±1.36[a]	1.22 ±0.04[a]
T-5	89.77 ±0.97[a]	1.22±0.02[a]
T-6	90.25 ±2.59[a]	1.19 ±0.02[a]

- T-1, T-2, and T-3 are single oil supplements of cod liver oil, sunflower oil and soybean oil respectively.
- T4, T-5 and T-6 are oil mixtures containing cod liver oil, sunflower oil and soybean oil at ratios of 2:1:1, 5:4:1 and 5:1:4 respectively.
- Means in the columns with different superscript letters are significantly different ($P<0.05$).

Table-53: Multiple linear regression analysis of fatty acid profile vs. SGR of diets supplemented with various lipid sources

Independent factor groups	R	R Square	Adjusted R Square	Std. Error of the estimate	Change Statistics				
					R Square change	F change	df1	df2	Sig. F change
Saturated	0.867	0.751	0.735	0.353	0.751	48.235	1	16	0.000
MUFA	0.861	0.741	0.725	0.360	0.741	45.712	1	16	0.000
PUFA	0.860	0.739	0.723	0.362	0.739	45.318	1	16	0.000
n3	0.859	0.738	0.722	0.362	0.738	45.074	1	16	0.000
n6	0.949	0.901	0.888	0.230	0.158	24.064	1	15	0.000
n3 HUFA	0.928	0.860	0.842	0.273	0.860	46.196	2	15	0.000
PREDICTOR(S)									
Saturated	14:0								
MUFA	20:1n11								
PUFA	18:2n6								
n3	22:6n3, 20:5n3								
n6	16:2 n6, 20:4n6								
n3 HUFA	22:6n3, 20:5n3								

Table-54: Multiple linear regression analysis of fatty acid profile vs. FCR of diets supplemented with various lipid sources

Independent factor groups	R	R Square	Adjusted R Square	Std. Error of the estimate	Change Statistics				
					R Square change	F change	df1	df2	Sig. F change
Saturated	0.879	0.773	0.758	0.032	0.773	54.393	1	16	0.000
MUFA	0.868	0.754	0.738	0.034	0.754	48.933	1	16	0.000
PUFA	0.937	0.877	0.861	0.024	0.115	14.008	1	15	0.002
n3	0.867	0.751	0.735	0.034	0.751	48.258	1	16	0.000
n6	0.966	0.933	0.924	0.018	0.158	35.415	1	15	0.000
n3 HUFA	0.899	0.808	0.782	0.031	0.808	31.533	2	15	0.000
PREDICTOR(S)									
Saturated	14:0								
MUFA	20:1n11								
PUFA	20:4n6, 18:2n6								
n3	22:6n3								
n6	20:4n6, 18:2n6								
n3 HUFA	22:6n3								

Table-55: Multiple linear regression analysis of fatty acid profile vs. PER of diets supplemented with various lipid sources

Independent factor groups	R	R Square	Adjusted R Square	Std. Error of the estimate	Change Statistics				
					R Square change	F change	df1	df2	Sig. F change
Saturated	0.845	0.713	0.695	0.060	0.713	39.788	1	16	0.000
MUFA	0.829	0.688	0.668	0.062	0.688	35.280	1	16	0.000
PUFA	0.906	0.821	0.798	0.049	0.122	10.237	1	15	0.006
n3	0.828	0.686	0.666	0.063	0.686	34.969	1	16	0.000
n6	0.949	0.901	0.888	0.036	0.120	18.187	1	15	0.001
n3 HUFA	0.888	0.789	0.760	0.053	0.789	27.983	2	15	0.000
PREDICTOR(S)									
Saturated	14:0								
MUFA	20:1n11								
PUFA	20:4n6, 18:2n6								
n3	22:6n3								
n6	20:4n6, 18:2n6								
n3 HUFA	22:6n3								

CHAPTER V
Discussion

5. DISCUSSION

From the age-old traditional aquaculture systems to the present day semi-intensive and intensive aquafarming systems, tremendous improvements in stock management, feed management and water quality management have lead to the augmentation of the aquaculture production (Bardach *et al.*, 1972; Pillay, 1992). In the process, to support the higher stocking rates and to sustain high productivity the feed management protocols have also evolved from the simple supplementary feeding of unprocessed or processed natural diets (New, 1980), to high end wholesome diets formulated to meet the nutritional demands of the species, to promote efficient food conversion, maximize growth performance, and to minimize food wastage. The survey of mud crab culture practices with thrust on feed management has opened up many untouched issues such as use of poor quality feeds, improper feeding schedules and feed management, and the constraints in handling storage and dispensing of fresh feeds, the possible environmental impact of the use of spoiled feeds etc. From the present study the acceptance of salted trash fish as the widely used feed in mud crab farms has been challenged because of its inferior nutritional profile and poor digestibility, resulting in lower SGR and PER coupled with high FCR. The superior nutritional profile of the combination diets, viz., better balanced amino acid and fatty acid profiles, higher ash (mineral) and crude fiber (chitin) levels has resulted in significantly higher growth response by juvenile *Scylla serrata* than the single feed stuffs like clam and fish. The energy budgeting trials of *Scylla serrata* juveniles fed fresh black clam meat at restircted ration could result in better assimilation efficiency and utilization of feed. The data on standard metabolic rate (SMR) and apparent specific dynamic action (SDA) at various moult stages indicate the marked differences among the moult stages, showing higher SMR at pre-moult, moult and post-moult stages; where as the SDA was high at intermoult stage consistent with the maximum feeding intensity exhibited by the juvenile crabs. The juvenile mud crabs (0.25±0.051 g) could be completely weaned on to the formulated pellet diets in a day or two. The results obtained from the dietary protein requirement trial show that for the very survival, juvenile crabs require

a dietary protein level above 0.25 g per g dry diet per day. Also the polynomial regression analysis suggest a higher protein requirement (0.45 g per g dry diet), and the higher protein requirement further points to a predominant carnivorous feeding habit of the juvenile crabs. The feeding trials with formulated diets having various lipid supplements clearly indicate the ability of the juvenile mud crabs to utilize plant oils partially along with marine oil sources in the diets, as there was no significant difference in the growth response of juvenile crabs fed diets supplemented with oil mixtures (one part marine oil : one part plant oil), as well as cod liver oil.

5.1. Survey of mud crab farms

From the survey, it is evident that there exists an increased diversification from shrimp farming to crab farming, but is hurdled mainly by lack of proper seed supply to meet increased demand. The dependence on fresh feeds limits the scope for further expansion of mud crab farming due to the lack of reliable and organized feed supply. Apart from that, the farmers face an uncertainty in the availability of fresh feeds due to the perishable nature and due to the seasonality in the availability. The lack of awareness about the feed quality has lead to the use of spoiled trash fish, with a concern to reduce the feed cost compromising the feed quality, leading to water quality issues. The heavy mortality associated with the transportation of cultured crabs during hot seasons may be attributed to the reduced stress tolerance of crabs associated with feeding, nutritionally inadequate, low quality feeds. Increased cannibalistic tendencies recorded in some farms may be due to lack of proper feed rationing and feeding schedules.

5.2. Evaluation of fresh and processed feeds

In the present study, the juvenile crabs were kept individually since they are known to exhibit territoriality and cannibalism (Keenan, 1999), there by ensuring the accurate assessment of survival and growth performance and feed efficiency. The survival rate of the crabs in all the treatments remained 100 % suggesting that the rearing conditions and the diets offered were adequate to sustain the crabs. Although cannibalism is a major cause of low survival of crustaceans during

laboratory rearing, this can be prevented with the addition of substrates (Kevrekidis and Kevrekidis, 1995). But use of substrate as well as hide-outs in the rearing troughs was not made in the present experiment as the same is likely to hamper the estimation of feed wastage and faecal matter.

The feeding habits of various crustaceans in nature show the preference for a wide range of feed items (Guillaume, 1990). Such feeding habit can be defined as an adaptation to meet the nutritional requirement of the animals (NRC, 1993). It has been generally accepted that the quantitative requirements of marine and brackishwater crustaceans for protein and fatty acids is better fulfilled by natural food of marine source (Deshimaru and Shigueno, 1972; New,1976). The diet of mud crabs in the nature includes molluscs, crustaceans, fish and dead animal matter with stray occurrence of plant-based materials (Hill, 1976 and 1979; Joel and Sanjeevaraj, 1986; Prasad and Neelakantan, 1988; Prasad *et al.*, 1988).

In the present study, when fresh and processed diets singly and in combinations were fed to juvenile *Scylla serrata*, the combination diets (SCF-1, 2 and 3) containing frozen shrimp head, frozen clam meat and frozen fish in three different ratios produced better growth performance in terms of higher SGR and PER and lower FCR indicating superior feed utilisation than single item diets suggesting that the combination feeds mostly meet the qualitative nutritional requirements of juvenile crabs. Studies with *Penaeus kerathurus* also revealed that a combination diet of natural unprocessed and un-supplemented diet of 43% anchovy and 33% shrimp head and 24% squid resulted in better growth performance than single item diets like mussel meat (Faranda *et al.*, 1984). In *Penaeus monodon* a feed combination of cooked cracked corn and gastropod meat produced better performance than those fed gastropod meat alone (Bombeo-Tuburan *et al.*, 1995) and in olive mud crab, *Scylla olivacea,* cooked cracked corn and gastropod meat gave the best performance (Rodriguez *et al.*, 2003).

Millamena and Quinitio (2000) showed that a mixed ration of a formulated diet in conjunction with natural food such as mussel meat, squid and trash fish resulted in improved reproductive performance and quality of seed produced in crab hatcheries and suggested that adequate supply of essential dietary nutrients in the formulated diets that are lacking in natural diets may have resulted in improved better broodstock performance.

There is scanty information regarding feed preference of *Scylla serrata* under captive conditions, though the wild crabs prefer to predate on molluscs, small crustaceans and rarely fin-fishes (Hill, 1976 and 1979; Joel and Sanjeevaraj, 1986; Prasad and Neelakantan, 1988). The observations on acceptability of various feeds provided to the juvenile crabs during the present study reveal a preference for fresh and frozen feeds by the juvenile mud crabs over the salted and dried ones.

It is generally believed that the natural diets preferred by the crustaceans in wild have superior digestibility over the processed and formulated diets (Lee and Lawrence, 1997; New, 1980). The apparent digestibility coefficients of the diets fed to juvenile *Scylla serrata* show that the fresh and frozen feeds are better digested than salted fish and dried clam meat. Besides, the proximate profile of the feeds used in the present study show that the combination diets have a better balance of nutrients particularly in terms of crude lipid, crude fiber, crude ash and nitrogen free extract. The crude ash and crude fiber contents in the diets represent the mineral and chitin contents respectively, and these are integral constituents of crustacean exoskeleton (Akiyama *et al.*, 1992). Growth in crustaceans happens as a result of moulting and a substantial proportion of minerals and chitin is lost in the moult shell, in spite of the re-absorption and synthetic capabilities (Strong and Daborn, 2002). To compensate the mineral loss, crustaceans resort to uptake from the aquatic environment (Deshimaru and Yone, 1978a; Kanazawa *et al.*, 1984) which is an active process, requiring energy expenditure which otherwise could be diverted for growth. Therefore it is presumed that the minerals and chitin from shrimp head in the

combination diets would have compensated the loss due to moulting enabling faster growth.

The juvenile crabs fed the combination diets also had the highest % dry matter content which is consistent with the higher deposition of the protein, minerals and chitin. The results are also highly correlated with the apparent digestibility of the diets.

The multiple linear regression (MLR) analysis of proximate components and amino acid and fatty acid profiles against SGR, FCR and PER excluded the non influencing factors and the predictive factors of response parameters were found out. Among the proximate components crude fibre was the most important factor influencing the SGR, FCR and PER which was followed by crude ash, crude protein and NFE.

The multiple linear regression analysis of amino acids show that all the essential amino acids have considerable influence on the SGR, FCR and PER. The superior balance of essential amino acids profile in the combination diets suggests their role in enhanced protein synthesis and efficient utilization of other nutrients resulting in tissue formation and higher growth response.

Among the nonessential amino acids proline and aspartic acid influenced SGR, where as FCR was influenced by proline and tyrosine and PER by proline and cystine. The primary function of nonessential amino acids in crustaceans, apart from being building blocks of protein synthesis, is energy production by catabolism and sparing action over essential amino acids by conserving them for protein synthesis (Guillaume, 1997). Here the energy required for growth and routine activities is mostly derived from nonessential amino acid catabolism, effecting better feed conversion and protein efficiency ratios. Proline, unlike other amino acids is not an important source of energy for crustaceans, and used only when the animal is under starvation (Brucet *et al.*, 2005), and being very important in osmo-regulation, it

is found in substantial quantities as free amino acid component of the crustacean haemolymph (McNamara *et al.*, 2004). Apart from that proline along with another non-essential amino acid glycine, has roles in regulating the contractile action of muscle fibres induced by zwitter ionic substances such as Ca^{2+} and other amino acids (Powney *et al.*, 2003). In crustaceans, free amino acids (FAA) have important roles in processes of moulting, growth, metamorphosis, anisosmotic regulation (i.e., osmoregulation) (Anger, 1996) and intracellular isosmotic regulation (maintenance of the osmotic equilibrium between cells and the hemolymph) (Haond *et al.*, 1999).) Studies on the involvement of free amino acids in osmoregulation of sea water adapted juvenile *Penaeus japonicus* (Dalla Via, 1986) show that the most abundant individual FAAs are glycine, taurine, arginine, proline and alanine. In crustaceans glycine, proline and alanine levels increase significantly in accordance with an increasingly saline environment (Mantel and Farmer,1983).

Among the tested diets the lowest performance in terms of SGR, FCR and PER was seen in juvenile crabs fed salted fish. The amino acid profile of the salted fish revealed significant reductions in the levels of EAAs such as isoleucine, leucine, lysine, threonine, phenylalanine and valine, besides NEAAs such as glycine and tyrosine as compared to that of frozen fish. Similarly the fatty acids profile, clearly indicate substantial degradation of PUFAs (18:2n6, 18:3n3 and 20:4n6) and HUFAs (20:5n3 and 22:6n3) in salted fish as compared to that of frozen fish. The reduction in amino acids and fatty acids content during storage conditions were explained by various authors in terms of the oxidative degradation of lipid and protein. Lipid oxidation is one of the major factors reducing quality and acceptability of meat and fat products (Morrissey *et al.*, 1998). The oxidation process involves the degradation of PUFA, vitamins and other tissue components and the generation of free radicals, which lead to the development of rancid odours and changes in colour and texture in food stuffs (Kanner, 1994). The iron from myoglobin and hemoglobin, hematin released from myoglobin, and non-heme iron contribute towards the iron induced oxidation of tissue lipids as a result of death and postmortem changes. Once free radicals such as lipid peroxides formed with the initiation of iron triggered

lipoxidation, it results in a chain reaction till tissue lipids get oxidized completely (Kanner, 1994).

The significant reduction in the essential amino acids such as isoleucine, leucine, threonine and phenylalanine may have affected their availability for protein synthesis and tissue buildup in salted fish. The superior response with frozen fish attributed to the intact essential amino acid profile because of frozen storage. The higher ash levels because of slat accumulation in the tissues of salted fish may also have affected the digestibility leading to poor utilization of slated fish compared to that of frozen fresh.

The better performance of fresh and frozen clam feeds compared to the frozen fish may be due to the higher lipid levels in frozen fish resulting higher energy levels resulting poor utilization of other nutrients (Sheen and Wu, 2000).

In the case of dry clam there was reduction in 20:5n3 and 22:6n3 contents, with 22:6n3 being significantly less. This may be attributed to the oven drying of the clam meat at temperatures above 50 °C in the presence of atmospheric oxygen resulting in the oxidative loss of otherwise highly unstable HUFAs. But no significant reduction in any of the amino acids was noticed in dry clam meat and may be attributed to the lower water activity in the dry clam meat, which is essential to initiate the lipid peroxide induced oxidation of proteins (Gardner, 1979). The superior growth response obtained with fresh clam compared to dry clam may be accounted for the reduced HUFA levels in dry clam, because of heat drying.

Protein oxidation mainly occurs via free radical reactions in which peroxyl radicals generated in the first stages of PUFA oxidation, and through the pro-oxidant activity of primary (hydroperoxides) and secondary (aldehydes, ketones) lipid oxidation products (Davis, 1995) abstracting hydrogen atoms from protein molecules, leading to the formation of protein radicals. The formation of non-covalent complexes between lipid oxidation products and reactive amino acids

residues, as well as the presence of certain metal ions, such as copper and iron, can also lead to protein radical generation (Goll *et al.,* 1964.). Lipid oxidation is thought to promote the oxidative damage of proteins, and Goll *et al.,* (1964) has presented data on the oxidative stability of proteins in animal products establishing close relationships between lipid and protein oxidation phenomena.

The most utilized saturated fatty acids and mono-unsaturated fatty acids were essentially the same as in all caridean species—16:0, 16:1n-7, 18:1n-9 and 18:1n-7 (D' Abramo, 1997).

The multiple linear regression analysis of fatty acid profiles indicated that among the saturated fatty acids, 16:0 was found to influence the FCR, where as saturated fatty acids did not influence SGR and PER. Among the mono-unsaturated fatty acids, 17:1n6, 20:1n11 and 16:1n7 were found to influence SGR, where as 17:1n6, 20:1n11 and 24:1n3 were found influencing the FCR, and PER was found to influence by 17:1n6 and 20:1n11. Saturated and mono-unsaturated fatty acids (MUFAs) are chiefly used as energy sources in lipid metabolism (D'Abramo, 1997).

Among the polyunsaturated fatty acids (PUFAs) arachidonic acid and linoleic acids were the most influencing fatty acids upon SGR and PER was influenced by all the PUFAs at lesser degree. No relation could be identified between FCR and PUFAs. Among the n3 fatty acids, 22:6n3, 20:5n3 and 24:1n3 have greatly influenced the SGR, where as PER by 22:6n3, 20:5n3, 24:1n3, and 18:3n3. Among n6 fatty acids, 20:4n6 and 17:1n6 were found to influence the SGR and PER, whereas FCR was influenced by 20:4n6 and 18:2n6. Both the n3 highly unsaturated fatty acids (HUFAs), viz., 22:6n3 and 20:5n3 were the most important to influence the SGR, FCR and PER.

PUFAs and HUFAs being the precursors for the eicosanoid metabolism have high EFA value for crustaceans (Kanazawa *et al.,* 1977a, 1978, 1979; D'Abramo and Sheen, 1993; Xu *et al.*, 1993, 1994).

5.3. Energy budget

Ingested energy of animals can be divided primarily into digestible and non-digestible (faecal) energies. The digestible energy comprises of assimilated and excreted fractions. In crustaceans, assimilated energy is channeled into metabolism, maintenance and production that include growth, ecdysis and reproduction (Mootz and Epifanio, 1974; Levine and Sulkin, 1979). The energy expended in metabolic processes, can be measured by respiration, and diverted for catabolism and maintenance of physiological functions including locomotion, feeding, food processing, and for the synthesis of new tissue (Kiørboe and Møhlenberg, 1987). Growth may be considered as the energy materially gained by the individual and can be stored as body reserves since moulted shell is accounted as energy loss (Mootz and Epifanio, 1974). The urinary excretion results in the release of nitrogenous compounds, viz. ammonia, urea, uric acid, purines, amino acids etc., that are primarily catabolic products of amino acids. The energy lost through catabolic processes may vary with the utilization of different diets by crustaceans, thus resulting in significant differences in the channeling of energy towards growth and in energetic efficiencies (Capuzzo, 1982).

In nature crabs such as *S. serrata* (Hill, 1976), *Carcinus maenas* (Walne, 1973; Elner and Hughes, 1978) *Portunus pelagicus* (Ebling *et al.,* 1964) and *Ovalipes punctatus* (Du Preeze, 1984) prey mainly on hard shelled molluscs particularly bivalves. Similar trend was also exhibited by lobsters, *Homarus americanus* (Ensis, 1973; Elner and Jamicson, 1979), *Panulirus homarus* (Berry, 1971; Smale, 1975), *Panulirus cygnus* (Joll and Philips, 1984) *Panulirus interruptus* (Shaw, 1986) and *Jasus lallandii* (Newman and Pollack, 1974; Pollock, 1979; 1982; Griffith & Seiderer, 1980)

The high assimilation efficiency (97.0 %) of the intake energy observed for juvenile *Scylla serrata* in the present study is comparable to that obtained in spiny lobster, *Panulirus homarus* (93.97 to 98.80 %, Radhakrishnan, 1989;

Vijayakumaran, 1990; Anilkumar, 2002), grass shrimp *Palaemonetes pugio* (97.8%, Vernberg and Piyatirativorakul, 1998), *Homarus americanus* (82 %, Logan and Epifanio, 1978), giant fairy shrimp *Brachinecta gigas* (>90 %, Daborn, 1978), of *Palaemonetes serratus, Pandalus platyceros, Penaeus setiferus, Penaeus aztecus* and *Metapenaeus bennetae* (97.0-98.0 %, Forster and Gabbot, 1971; Condrey *et al.,* 1972; Moriarty, 1977) fed various fresh feeds. In *Macrobrachium lamarrei,* Marian *et al.* (1986) reported assimilation efficiencies of 88 % and 94 % when fed goat liver and tubifex respectively.

The energy loss through moult production (7.94 % of consumed energy) in juvenile *Scylla serrata* observed in the present study is found to be higher than those reported in *Panulirus homarus* (3.2-3.9 %, Anilkumar, 2002) and *Penaeus vannamei* (<3%, Cousin, 1995). Radhakrishnan (1989) and Vijayakumaran (1990) reported a loss of 27.3-32.7% of the converted energy. In the present study it was above 45 % of converted energy. The high moult loss in juvenile *Scylla serrata* may be accounted for the harder exoskeleton of crabs in general, resulting a heavier loss.

The pattern of nitrogen excretion is typical to that shown by aquatic invertebrates (Prosser and Brown, 1961) with NH_3 being the principal excretory product. Apart from NH_3, urea, uric acid, amino acids and purines are also found in the excretion of marine and brackish water crustaceans (Parry, 1960; Pandian, 1975). The rate of total ammonia excretion by the juvenile crabs in the present experiment (1.71 % of intake energy, 25-30 °C, 28 ppt) is found to be almost half of that reported for *Panulirus homarus* (3.35 % of intake energy, Radhakrishnan, 1989; Vijayakumaran, 1990; Anilkumar, 2002) at 24-28 °C and 32-34 ppt salinity.

In the present study a mean oxygen consumption of 0.33 ml O_2 /g/hr (23.6 ±3.5 °C, 28 ±1 ppt) was obtained for juvenile *Scylla serrata*, where as in *Panulirus homarus*, a tropical crustacean, the mean oxygen consumption lies with in the range of 0.27-0.53 ml O_2 /g/hr (24-28 °C, 32-34 ppt) (Radhakrishnan, 1989; Vijayakumaran, 1990 and Anilkumar, 2002) . But the mean oxygen consumption rate

of juvenile *Scylla serrata* is found to be lower than that of temperate crustaceans such as *Homarus americanus* (0.036 O_2/g/hr at 5 °C, Mc Leese & Watson, 1968) *Homarus gammarus* (0.068 O_2/g/hr, at 15°C, Jollyet and Regnard, 1877), *Austropotamobius leptodactylus* (0.07 O_2/g/hr, at 19-21°C, Bishop, 1950) and *Procambrus clarki* (0.066 O_2/g/hr, at 25°C, Davison, 1956).

Barakai and Branch (1988) estimated the metabolic expenses as 81 % of the total intake energy in *Jasus lalandii.* In *Panulirus homarus* (Anilkumarkumar, 2002) the metabolic expense is calculated as 84.15 % of total intake energy when fed fresh clam at 15% of body weight. In the present study with *Scylla serrata* juveniles, metabolism was about 79-80% of the total intake energy when fed fresh clam meat at 7.8 % of body weight. The reduced expenditure for metabolism in the present study is perhaps due to the restricted ration offered. In the former study (Anilkumar, 2002) it's likely that over feeding had resulted in excessive nutrient intake and hence the higher metabolic rate. Marian *et al.* (1986) observed 80.2, 86.2, and 79.5 % of metabolism in *Macrobrachium lamarrei* fed Tubifex, goat liver and carrot, indicating the variation in metabolic rate associated with feed stuffs and their nutritional profile.

The FCR obtained for the present study is comparable to that obtained by Crear *et al.* (2000) in *Jasus edwardsii* fed mussel (1.26) and prawn (2.24), but lower than those fed formulated feed (3.78). In *Panulirus homarus* when fed with fresh mussel, clam and a mixture of these with fish had FCR of about 2.4, where as when fed fish alone resulted in higher FCR of 8.3 (Anilkumar, 2002). Radhakrishnan (1989) and Vijayakumaran (1990) reported FCR of 2.7-4.1 when fed mussel for the same species and in *Panulirus ornatus, fed* mussel, utilsed the feed with 4.7 FCR.

5.4. Dietary protein requirement

Crustaceans like other animals need protein in the form of essential and non-essential amino acids for maintenance of life, growth and reproduction. The protein requirement depends on animal characteristics (species, genetic make up, physiological state, age, size etc.) quality of protein (digestibility and biological

value), dietary energy, non-energy nutrients and environmental characteristics. The optimal protein requirement is almost independent of environmental parameters, but dependant on size and age, where as true protein requirement varies widely with the developmental stage of animal and affected by environmental parameters (Guillaume, 1997).

In the present study diets were formulated considering the general requirements of crustaceans (Akiyama *et al.,* 1992; National Research Council, 1993; D'Abramo and Sheen, 1994; Davis and Gatlin, 1996) and the major ingredients include fish meal, clam meal, shrimp meal, squid meal, soybean flour and wheat flour. The water stability (> 4 hours) and energy content (17.14 -17.56 kJ/g) of test diets containing graded protein levels also met the recommended values by Cuzon and Guillaume (1997).

The crabs were reared individually as mentioned earlier, to avoid cannibalism. From the results it is evident that the juvenile crabs fed isocalorific and isolipidic diets containing 40.5 % crude protein with a lipid content of 8.52 % showed the best growth response and that high protein (50% crude protein) and low protein (<35 % crude protein) diets produced relatively poor response. The optimum protein requirement of the juvenile mud crab was further estimated to be 43.7-45.2% using third degree polynomial fit. The results were comparable to the performance of larger juveniles of *Scylla serrata* (11.18± 0.66 g) fed 40% crude protein as obtained by Catacutan (2002). The slight increase in protein requirement estimated using third degree polynomial fit (43.7-45.2%) may be attributed to the use of smaller juveniles (0.25±0.051 g) in the present study, where as the earlier author has used larger specimens (11.18±0.66g), and from the study it is evident that the younger animals require slightly higher protein content in the diet to support faster growth rates. Besides the difference in dietary protein source and digestible energy values may also have contributed to the marginal difference (Smith *et al.*, 1985). Millamena and Quinitio (2000) were able to mature and spawn adult *Scylla serrata* fed formulated feed containing 46.03% crude protein and 11.64% lipid level, but

performance was superior when fed it in conjunction with natural feeds (squid, fish by-catch, mussel etc.). Similar results were obtained by Djunaidah *et al.* (2003) in *Scylla paramamosain* fed formulated diets having 40.4-43.3% crude protein and 15.6-17.9% crude fat.

The crabs fed lower protein levels 15 % and 20 % resulted in cent percent mortality in the first moulting after the feed trials commenced. This clearly indicates a higher dietary protein level above 20 % is required by the juvenile crabs for minimal maintanance.

It is also evident that a reduction in the protein level from 50.53% to 40.50% and consequent increase in non-protein energy in the present study did not make any significant difference in FCR but the WG, SGR and PER improved significantly. These results indicate that energy from non-protein sources spared protein utilization in this species. Such protein-sparing action has also been reported in other crustaceans, for example, *Penaeus monodon* (Shiau and Peng, 1992), *Homarus americanus* (Conklin, 1995) and *Eriocheir sinensis* (Mu *et al.*, 1998).

SGR, PER and body protein contents of the juvenile crabs in the present study tended to increase as the dietary protein increased from 25.37 to 40.50 % and observed to decline thereafter and an inverse trend was observed for FCR. This is possibly due to the increase in feed consumption by crabs to derive adequate levels of dietary protein to meet the metabolic and growth needs. Because of the iso-caloric nature of diets and protein-sparing action of non-protein nutrients, crabs fed diets with lower protein levels may have efficiently utilized the dietary protein to grow. The decline observed in growth at higher protein level may be ascribed to the catabolism of excess dietary protein for energy production. Koshio *et al.* (1993) found that the ammonia excretion of *Penaeus japonicus* increased as dietary protein increased, which indicated that excess protein given in the feed was catabolized for energy purpose, once reaching the limit of body protein deposition.

The P / E ratio that provided the best SGR, FCR and PER was found to be 22.68 mg/kJ for *Scylla serrata* juveniles fed the diet containing 40.53 %. This ratio was very close to the optimum P/ E of 20.5-27.5 mg/kJ obtained by Catacutan (2000) for the same species. Higher P/E ratios of 29.88 mg/kJ for juvenile *P. monodon* (Shiau and Chou, 1991), 26.29-31.07mg/kJ for *P. merguiensis* (D'Abramo and Sheen, 1994) and 27.25–29.39 mg protein/kJ for juvenile *A. astacus* (Ackefors *et al.*, 1992) were reported by various investigators.

The dry matter content and proximate profiles of juvenile crabs were also significantly affected by the dietary protein level in this study. The direct relationship between dietary protein and body protein noticed in this study further conforms to the observations of Chen *et al.* (1994) in juvenile *Eriocheir sinensis,* and Alava and Lim, (1983) in juvenile *Penaeus monodon.* The body lipid, carbohydrate, crude ash and gross energy level also revealed a direct relationship with dietary protein levels. Protein being the major component of animal tissues, the optimal availability of dietary protein ensures proper growth in animals by tissue formation, incorporating lipids, minerals and other minor components in the required ratios (Lehninger *et al.*, 1991).

From the results of the study and the facts mentioned above, it is concluded that *Scylla serrata* early juveniles (0.25±0.051 g), being oriented to a carnivorous feeding habit require a dietary protein level of 43.70-45.20% along with 8.52% lipid and gross energy value of 17.14 -17.56 kJ/g diet and that sub-optimal and exess protein levels lead to poor growth performance. It is also evident that the juveniles require a minimum dietary protein level exceeding 25 % for normal moulting and survival as protein deficiency led to complete mortality.

5.5. Evaluation of lipid supplements in formulated diets

Dietary lipids play an important role as a source of energy, phopholipid and essential fatty acids for crustaceans. Dietary lipid requirement in crustaceans may be influenced by a number of factors: the quality and quantity of dietary protein, the

quality, quantity, and availability of other energy sources, and the quality of the dietary oil (D'Abramo, 1997). The nutritive quality of lipids primarily depends upon their fatty acids profile, especially the essential fatty acid groups such as PUFAs and n3 HUFAs (Kanazawa, 1995). PUFAs and HUFAs from the n-3 series, especially DHA and EPA, have been identified in the last three decades as essential nutrients for marine animals (Rainuzzo *et al.*, 1997; Sargent *et al.*, 1999).

The growth response obtained in the present study clearly indicate that the juvenile crabs fed diets supplemented with mixtures of marine and plant lipids performed equally well as that of crabs fed diet, supplemented with marine lipids alone. The poor response in juvenile crabs to diets supplemented with plant oils alone is mainly due to the deficiency of n3 HUFAs, viz, 20:5 n3 and 22:6 n3. The survival was not however affected in these treatments, as the fatty acid analysis of the basal diets showed moderate levels (20:5 n3 at 0.20 % and 22:6 n3 at 2.06-0.22 %) of these n3 HUFAs. The HUFAs contributed by the lipid in the basal dietary marine ingredients mix (fish meal, shrimp meal, squid meal and clam) could satisfy the basic maintenance requirement, but not in adequate quantities to support maximum growth response.

The evaluation of various lipid supplements individually and in combinations for *Penaeus indicus* showed that a mixture of plant oil and fish oil gave the best growth response (Chandge and Paulraj, 19907) and attributed to its balance in PUFAs and HUFAs. Lim *et al.* (1997) evaluated the growth response and fatty acid composition of juvenile *Penaeus vannamei* fed different dietary lipids and found that menhaden oil, rich in n-3 HUFA, was the most nutritious for *Penaeus vannamei*, and among plant oils, those rich in 18:3n3 had a higher nutritional value than those rich in18:2n6.

In the present study, 18:2n6 and 18:3n3 were present at 0.97-4.40 % and 0.34-0.54 % respectively. And this is found to satisfy the requirement of the juvenile mud crabs in the present study. In spite of having higher 18:2n6 and 18:3n3 levels in

plant lipid supplements in the present study, the poor growth further confirms the inability of mud crabs to bio-convert C-18 PUFAs into n3 HUFAs (Suprayudi *et al.*, 2004) and less EFA value for 18:C PUFA as compared to that of n3 HUFAs. A series of experiments (Kanazawa *et al.*, 1977, 1978, 1979b; Xu *et al.*, 1993, 1994) have demonstrated a greater nutritive value of n3 HUFA relative to PUFAs in *Penaeus japonicus* and *Penaeus chinensis.*

The study also indicated that the lower level of n3 HUFAs in the 1:1 fish oil-plant oil mixtures (1.10-1.11 %) than in fish oil (1.81 %) did not affect the growth performance of the juvenile crabs, confirming that the diets supplemented with fish oil-plant oil mixtures provided optimum n3 HUFA levels to the juvenile mud crabs ensuring best growth response and feed efficiency.

In the present study the 0.69-0.70% (DM basis of diet) of 22:6n3 and 0.41 % of 20:5n3 in the diets supplemented with fish oil-plant oil mixtures was found sufficient to promote better growth response as against the fish oil supplemented diets having 1.18% of 22:6n3 and 0.63% of 20:5n3.

The levels of PUFAs (3.22-3.27 %) and HUFAs (1.10-1.11 %) in 1:1 fish oil-plant oil mixture supplemented diets for mud crab juveniles are much higher than the PUFA level of 0.5 to 1% proposed by Kanazawa *et al.* (1979a) for *Penaeus japonicus*, 1% tridocosahexaenoin proposed by Xu *et al.* (1993, 1994) for *Penaeus chinensis* and 0.2 % of tri-docosahexaenoin and tri-eicosapentaenoin for juvenile *Scylla serrata* reported (0.2%) by Sheen and Wu (2003), but it is lower than the 2.5% PUFA and 1.5 to 2.0% HUFA proposed by Merican and Shim (1997) for *Penaeus monodon*. Essential fatty acid levels in the best performed diets of juvenile mud crabs in the present study were in consensus with the general EFA requirement of around 1% for crustaceans (D'Abramo, 1997).

The HUFA levels which gave the best growth response in juvenile *Scylla serrata* (0.2 % of tri-docosahexaenoin and tri-eicosapentaenoin) reported by Sheen and Wu (2003), was at the same levels in the poorly performed plant oil supplemented diets used in the present study.

Saturated fatty acids are non essential fatty acids and can be synthesized *de novo* or obtained by desaturation of MUFA and HUFA (Sargent, 1995). In the present study the influence of saturated and unsaturated fatty acids auch as, 14:0, 17:0, and 14:1n7, 20:1n11 and 22:1n11 on the growth response of juvenile *Scylla serrata* confirms their importance as energy sources in the lipid metabolism of crustaceans (D'Abramo, 1997; Sargent *et al.*, 1999; Gonza´lez-Fe´lix *et al.*, 2002).

Among the PUFAs, greater influence on growth is effected by 20:4n6. The quantity of 20:4n6 in the diets was significantly lesser than that of other EFAs but did not vary significantly among the diets. Both the HUFAs, viz. 22:6n3 and 20:5n3 had prominent influence on growth and moulting in the present study. Crustaceans require n3 HUFAs for maximum growth, feed efficiency, and survival (Lim *et al.*, 1997). 20:4n6, 22:6n3 and 20:5n3 are known precursors of eicosanoid metabolism, playing an important role in moulting and growth (Rowley *et al.*, 1995). Molting of crustaceans and insects is regulated by ecdysteroids secreted by Y-organ located on the eyestalk, which up on release into haemolymph, directly regulates molting (Subramoniam, 2000). Doston *et al.* (1993) reported that [3H]-ecdysone was metabolized into three different compounds in the embryos and larvae of *Ornithodoros moubata* and one of them conjugated with a C-22 fatty acid like DHA regulating the moulting by modulation of ecdysteroid metabolism. Apart from being precursors in eicosanoid metabolism 20:4n6 and 20:5n3 are important as structural components of cell membranes playing a key role in membrane formation and growth (Bell and Sargent, 2003). DHA is present in extremely high amounts in the brain and ophthalmic structures, indicating that it may play an important role in the development of the central and sensory nervous system of crustaceans (Bell and Dick, 1990; Fox et al., 1994). The SGR, FCR, PER, carapace width gain, moulting frequency and proximate profile of the juvenile crabs at the end of the present study clearly indicate the importance of essential fatty acids such as a20:4n6, 22:6n3 and 20:5n3 in the diet of juvenile mud crabs.

The lower n3/n6 ratios in diets supplemented with plant oil alone and fish oil-plant oil mixtures is primarily due to the very high content of 18:2n6 in plant oils which in turn resulted in higher percentages of 18:2n6 (0.97-4.40%, DM basis) in diets. Higher n3/n6 ratio was found in fish oil supplemented diet (1.76) comparable to those reported by Wen *et al.* (2002) in the diets formulated with marine ingredients.

The DHA/EPA ratio was nearly 2 in the diets supplemented with fish oil alone and mixtures of fish and plant oils which gave the highest growth response. These ratios were comparable to the calculated ratios of best performed diets for *Penaeus vannamei* (Gonza´ lez-Fe´lix *et al.*, 2002).

Conclusion & Summary

CONCLUSION

- Salted trash fish the most widely used feed in fattening is found to be inferior in terms of amino acids and fatty acids composition and poor digestibility and less attractive to the juvenile crabs than the other feeds.
- Frozen stored and fresh feeds are found to be more digestible than salted and dried feed stuffs.
- The combination diets (SCF-1 -1: 2: 7, SCF-2 - 1: 4: 5 and SCF-3 - 1: 5: 4 of frozen shrimp head (S), frozen clam (C) and frozen fish (F)) were found to be superior in terms of amino acids and fatty acids profile. These diets also had more crude fibre in the form of chitin and mineral in the form of crude ash, from the shrimp heads, which in turn would have supported the higher mineral and chitin requirement to compensate their loss through moult. The combination diets were more attractive to crabs than other feeds, which may be attributed to the higher free amino acids and peptide leaching from the mid-gut gland of shrimp heads as a result of autolysis.
- The energy budget studies indicate that the juvenile crabs about 79.20-80.12 % of the daily intake energy for the metabolic expenses where as only 9.32 % was converted to tissue growth. The moult loss is accounted for a massive 7.94 % of intake energy unlike in shrimps and lobsters (3.0-3.9 %).
- The better assimilation efficiency and conversion efficiency showed by the crabs may be attributed to the restricted of feed ration, unlike satiation level reported by most workers
- The estimated metabolic rates during the three different moult stages vary greatly and the same is further confirmed by the feeding intensity over the moult cycle.
- The intake energy (272.46 kJ/g mid-body wt./day) during the intermoult period is 495.92 % of the pre-moult intake (54.94 kJ/g mid-body wt./day) and 148.78 % of moult and post-moult stages (183.13 kJ/g mid-body wt./day) with intermoult apparent specific dynamic action (ASDA, 76.76 kJ/g mid-body

wt./day) as 1090.34 % of pre-moult ASDA (7.04 kJ/g mid-body wt./day) and 144.53 % of moult and post-moult ASDA (53.11 kJ/g mid-body wt./day).

- Standard metabolic rate of moult and post-moult (SMR, 207.89 kJ/g mid-body wt./day) is about 309.36 % of intermoult SMR (67.20 kJ/g mid-body wt./day) and 133.98 % of pre-moult SMR (155.17 kJ/g mid-body wt./day).
- The nocturnal feeding intensity observed in crabs is in conformity with the information available for crustaceans in general, with two peak feeding phases over 14 hrs period between 1700 hrs to next day 0700 hrs. The highest feeding intensity for a shorter duration of 2 hrs was found immediately after the feed offered, ie, between 1700 hrs and 1900 hrs and another less intense peak was found spanning over a longer duration, between 2200 hrs and 0500 hrs.
- The trials with the formulated diets clearly indicate that the juvenile crabs can be easily weaned to artificial diets supporting normal growth and moulting.
- The protein requirement study indicates that the juvenile crabs require relatively protein levels (above 25 %) for the minimal metabolic maintenance.
- The polynomial estimate shows a higher protein requirement of about 45 % for the best growth performance which suggests the carnivorous status of the crab juveniles
- 1:1 ratio of cod liver oil and PUFA rich plant oil such as sunflower oil and soybean oil mixtures could very well support a comparable growth performance obtained with diets supplemented with marine lipid alone.
- The results indicate that mud crabs like shrimps can efficiently utilize plant lipids rich in PUFAs as a partial supplement to marine oils.
- This can to some extent bring down the cost of formulated diets.
- The information generated from the present study would be useful in the formulation of nutritionally adequate, off-the- shelf, eco-friendly pellet feeds for mud crab fattening and grow-out as well as for improved feed management practices in culture.

SUMMARY

Among the crustacean candidate species for aquaculture, mud crabs of the genus *Scylla* are gaining importance as alternative to tiger shrimp especially in the Asian scenario, in the context of consecutive white spot disease outbreaks rendering shrimp culture, a risky affair. Literature currently available on mud crab culture has scanty information on the nutritional requirement of the species, and little scientific basis on feed management. Currently a number of locally available fresh or semi-processed feedstuffs including kitchen left-over and animal entrails are used globally for mud crab aquaculture. The emerging scenario of mud crab culture demand scientifically designed fresh and processed feed combinations and formulated off the shelf-diets.

The survey on mud crab farming made during the study clearly indicate the least concern on the feeds used and feed management. Currently farmers widely use fresh, salted and dried trash fish being a cheap source, irrespective of the quality. Feeding with fresh and cooked animal entrails was also practiced by some farmers. The use of fresh and cooked molluscan meat like clam meat was recorded rarely. The evaluation of currently used feeds and their combinations were done with a view to identifying the best feeds among the presently used feeds to maximize the feed utilization thereby reducing the wastage and to support faster growth rates of mud crabs. The studies on energy budget were aimed at gathering information on energy requirements and utilization at various moult stages and to optimise feed rationing and maximize feed utilization. Feeding trials with pellet diets were conducted to determine the protein requirement and to evaluate various diets supplemented with different lipid sources singly and in combination. The information generated from the studies on energy budget, protein requirement and evaluation of lipid sources can be used in formulating nutritionally adequate, eco-friendly, off-the shelf pellet feed for mud crab culture.

Summary of the findings from the studies are given below:

- The most common feeds currently fed to crabs by the farmers for grow-out and fattening were trash fish stored in brine; boiled clam meat and slaughterhouse waste, and rarely fresh trash fish and salt-dried trash fish.
- Indiscriminate feeding with spoiled and poor quality feeds available at the lowest price was seen in most of the farms.
- Spoiled fish procured and stored repeatedly in the same brine solution was the most commonly used feed in the farms, which was less attractable to crab. As a result feed wastage and water quality spoilage were noted.
- Lack of awareness among the farmers on the importance of quality and proper storage of feeds, feeding strategies and feed management was noted.
- Loss of stock due to the escapement and cannibalism was common as a result of untimely feeding schedules and insufficient feed rations.
- During the summer months (April-May) high mortality rates were seen in the farms, and harvested crabs during the transportation due to excessive heat, which may be attributed to the poor health condition of the stock, which in turn could be a result of poor quality feeds and improper feed management.
- The quality of feeds and feeding schedules were seen compromised for the cost, availability and convenience.
- Among the various fresh and processed diets, the combination diets promoted superior growth in terms of SGR (1-1.13%) FCR (1.17-1.27), PER (1.45-1.52), percentage wet (81.52-85.20 %) and dry (99.18-103.07 %) weight gains and the carapace width gain (24.30-25.93 %) with shorter intermoult duration.
- The proximate composition of experimental crabs showed better nutrient deposition in juvenile crabs fed the combination diets (DM=29.61-30.29 %, protein= 11.45-11.56%, crude fiber=4.06-4.91, crude ash=10.46-10.75 %, gross energy=2.78-2.81 kJ/g)
- The crabs fed salted fish (DM=20.72 %, crude protein= 6.17 %, crude fiber=2.90 %, crude ash=5.11 %, gross energy=2.64 kJ/g) and dry clam (DM=21.02 %, crude protein= 8.34 %, crude lipid=1.32 %, crude fiber=3.26 %,

crude ash=6.23 %, gross energy=2.64 kJ/g) showed inferior proximate profiles than those fed other diets.

- The amino acid profiles of fresh and processed feeds reveals that the salted fish has the lowest levels of essential and non essential amino acids (21.05 % and 20.46 % respectively) with the highest NPN content (15.88 %). Significant reduction in essential amino acids such as isoleucine, leucine, lysine, threonine and phenylalanine as well as non-essential amino acids such as cystine, glutamic acid, glycine and proline were found in salted fish than frozen fish.
- The highest content of saturated fatty acids was observed in salted fish (13.56%). The ratio of saturated/unsaturated fatty acids was lower in the frozen fish and combination diets (0.77-0.82), where as salted fish had the highest saturated/ unsaturated fatty acid ratio (1.61).

- Relatively high n3/n6 ratio was found in frozen fish (6.51 %) followed by the combination diets (2.90-3.72), while the lowest ratio was recorded in dry clam (1.60).
- Linoleic acid (18:2n6) levels fluctuated between 0.22 and 0.61 % of the total fatty acids in the feeds with the highest levels in frozen fish (0.61%) followed by salted fish (0.46 %), combination diets (0.38-0.44 %) and the lowest in clam feeds (0.22 %).
- Frozen fish had the highest content of linolenic acid (18:3n3, 1.73 %) followed by combination diets (1.02-1.33 %) and the lower levels were recorded in salted fish (0.69 %) and clam feeds (0.63-0.64 %).
- Combination diet SCF-1 had the highest arachidonic acid content (20:4n6, 0.39 %) followed by frozen fish (0.31 %) and very low levels of the same were recorded in the clam feeds (0.03-0.06%).
- The highest EPA (20:5n3) content was found in frozen fish (2.16 %), followed by combination feeds (0.78-1.26 %) and clam feeds and salted fish had the lowest EPA content (0.15-0.54 %).

- DHA (22:6n3) level was also high in frozen fish (2.46 %) followed by the combination diets (1.49-2.07 %) and the lowest level was recorded in salted fish (0.18 %). DHA/EPA ratios in the combination diets ranged from 1.64 to 1.91. The lowest DHA/EPA ratio was found in salted fish (0.33) where as clam feeds (3.07-4.70) had the highest ratios among the feeds.
- Out of the total energy intake (197.57 J/g mid body wt./day) 80.12 % (158.28 J/g mid body wt./day) is spent towards the metabolism, 1.71 % (3.39 J/g mid body wt./day) towards excretion, 7.94 % (15.69 J/g mid body wt./day) towards the exuviation and about 9.32 % (18.41 J/g mid body wt./day) is deposited in tissues.
- The standard metabolic rate was highest at moult and post-moult stages (207.89 ±0.42 J/g/day, 88.00 ±0.16 % of total metabolism-TM), followed by pre-moult stage (155.17 ±0.21 J/g/day, 95.66 ±0.14 % of TM) and the lowest was observed at intermoult stage (67.20 ±0.10 J/g/day, 46.68 ±0.12 % of TM).
- The highest apparent specific dynamic action (ASDA) recorded at intermoult stage (76.76 ±0.16 J/g/day, 53.32 ±0.19 % of TM) corresponding to the peak feeding intensity of the juvenile crabs, followed by the post-moult stage (53.11 ±0.35 J/g/day, 22.48 ±0.11 % of TM) and the lowest SDA was recorded at pre-moult stage (7.04 ±0.15 J/g/day, 4.34 ±0.02 % of TM), associated with lowering of the feeding intensity.
- From the results the highest TM was observed at post-moult and moult and post-moult stages (236.25 ±0.37 J/g/day, 129.00 ±3.12 % of daily intake energy) followed by pre-moult stage (162.06 ±0.25 J/g/day, 295.27 ±1.93 % of daily intake energy) and the lowest at intermoult stage (143.96 ±0.42 J/g/day, 52.84 ±1.82 % of daily intake energy).
- Observations on the feeing rates over the moult cycle showed that the highest feeding intensity was during the intermoult period; where as during the pre-moult stage rate of feeding declines reaches near zero intensity. No feeding was seen on the days prior to and after moulting and reached the normal feeding intensity in 2-3 days after moulting.

- The highest feeding intensity for a shorter duration of 2 hrs was found immediately after the feed was offered, ie., between 1700 hrs and 1900 hrs and another less intense peak was found spanning over a longer duration, between 2200 hrs and 0600 hrs.
- At 15 and 20 % crude protein levels in the diet, 100 % mortality was observed at the first moult during the feeding trial, where as in all other treatments 100 % survival was noted.
- Significantly higher growth (SGR-3.81-4.37 %, FCR-1.86-1.98 and PER-1.01-1.31, wet wt. gain=1615-1993 %, dry wt gain=1638-2001 %, carapace width gain =194-223 %)) was noticed in CP-40, CP-45 and CP-50 (40, 45 50 crude protein levels) along with shorter intermoult duration (10 days/moult).
- Diets CP-25, 30 and 35 (25%, 30 % and 35 % crude protein levels) showed poor digestibility (62.25-79.66 %) as compared to CP-40, 45 and 50 (88.97-89.79 %).
- The proximate composition showed a higher deposition of nutrients in crabs fed diets CP-40, CP-45 and CP-50. Among these three, CP-40 gave the best response in terms of growth and nutrient deposition.
- But the polynomial regression of growth data against dietary protein levels projected an optimum protein requirement of about 45 % for the best growth response in juvenile crabs.
- The growth response obtained by the juvenile mud crabs fed diet T-1 (cod liver oil supplemented), T-4, T-5 and T-6 (oil mixture supplemented- cod liver oil, sunflower oil and soybean oil at 5:2.5:2.5, 5:4:1, 5:1:4 respectively) was found superior (SGR-3.85-3.98 %, FCR-1.19-1.22 and PER-1.99-2.02, wet wt. gain=1466-1470 %, dry wt gain=1435-1454 %, carapace width gain =110 % and intermoult duration=10.30-10.33 days/moult) to the crabs fed diets T-2 (sunflower oil supplemented, SGR-2.49 %, FCR-1.35, PER-1.75, wet wt. gain=675.51 %, dry wt gain=208.22 %, carapace width gain =93.28 %, intermoult duration=13.71days/moult) and T-3 (soybean oil supplemented, SGR-2.55 %, FCR-1.33 and PER-1.80, wet wt. gain=442.69 %, dry wt

gain=155.58 %, carapace width gain =102.13 %, intermoult duration=13.38 days/moult). The apparent digestibility coefficient of feeds T-2 (85.63 %) and T-1 (86.47 %) were inferior to other diets (89.77-90.25 %).

- Though diets T-2 and T-3 showed a higher PUFA content (4.83-5.13 %), both n3 (0.76-1.25 %) and n3 HUFA contents (0.42 %) were substantially lower in T-2 and T-3 than that observed in other diets.
- Diet T-1 (cod liver oil supplemented) showed the highest n3 HUFA content (1.81 %), where as diets having mixed oil supplements had moderate n3 HUFA content, which ranged from1.10 to 1.11 %.
- Diets T-2 and T-3 had the highest 18:2n6 levels (4.21-4.40 %), where as in diets T-4, T-5 and T-6 the levels fluctuated between 2.62 and 2.68 % of the total fatty acids in the diets. Diet T-1 supplemented with cod liver oil recorded the lowest 18:2n6 levels (0.97 %).
- Diet supplemented with soybean oil (T-3) was found to have the highest 18:3n3 content (0.82 %), followed by diets supplemented with oil mixtures (T-4, T-5 and T-6; 0.40-0.54 %) and soybean oil (T-2; 0.34 %).
- Arachidonic acid (20:4n6) in the various diets did not show any significant difference ranging from 0.10 to 0.11 %.
- Diet supplemented with cod liver oil (T-1) had the highest EPA (20:5n3) content (0.63 %) followed by diets T-4, T-5 and T-6 (0.41 %) and diets T-2 and T-3 had the lowest EPA content (0.20 %).
- The highest DHA (22:6n3) content (1.18 %) was recorded in diet T-1 (cod liver oil supplemented) and the diets T-2 and T-3 had the lowest of DHA level (0.22 %). Diets supplemented with oil mixtures had moderate levels of DHA (0.69-0.70 %).
- DHA/EPA ratio was relatively higher (1.81) in T-1, followed by T-4, T-5 and T-6 (1.68-1.71).

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APPENDIX

ANOVA TABLES

Growth response of juvenile *Scylla serrata* fed fresh and processed feeds

		Sum of Squares	df	Mean Square	F	Sig.
SGR	Between Groups	4.287	7	.612	227.880	.000
	Within Groups	.108	40	2.688E-03		
	Total	4.395	47			
FCR	Between Groups	132.079	7	18.868	973.786	.000
	Within Groups	.775	40	1.938E-02		
	Total	132.854	47			
PER	Between Groups	10.467	7	1.495	67.011	.000
	Within Groups	.893	40	2.231E-02		
	Total	11.360	47			
WETWG	Between Groups	12606.397	7	1800.914	55.210	.000
	Within Groups	1304.777	40	32.619		
	Total	13911.174	47			
DRYWG	Between Groups	62802.175	7	8971.739	632.745	.000
	Within Groups	567.163	40	14.179		
	Total	63369.338	47			
CWG	Between Groups	1873.129	7	267.590	73.440	.000
	Within Groups	145.747	40	3.644		
	Total	2018.876	47			
IMD	Between Groups	26909.250	7	3844.179	1601.741	.000
	Within Groups	96.000	40	2.400		
	Total	27005.250	47			

Proximate composition of juvenile *Scylla serrata* fed fresh and processed feeds

		Sum of Squares	df	Mean Square	F	Sig.
M	Between Groups	304.598	7	43.514	129.130	.000
	Within Groups	5.392	16	.337		
	Total	309.989	23			
DM	Between Groups	304.598	7	43.514	129.130	.000
	Within Groups	5.392	16	.337		
	Total	309.989	23			
CP	Between Groups	82.154	7	11.736	199.767	.000
	Within Groups	.940	16	5.875E-02		
	Total	83.094	23			
EE	Between Groups	19.650	7	2.807	182.628	.000
	Within Groups	.246	16	1.537E-02		
	Total	19.896	23			
CF	Between Groups	8.663	7	1.238	21.085	.000
	Within Groups	.939	16	5.870E-02		
	Total	9.602	23			
CA	Between Groups	109.152	7	15.593	46.237	.000
	Within Groups	5.396	16	.337		
	Total	114.548	23			
NFE	Between Groups	9.107	7	1.301	84.641	.000
	Within Groups	.246	16	1.537E-02		
	Total	9.353	23			
GE	Between Groups	1.080	7	.154	50.701	.000
	Within Groups	4.868E-02	16	3.043E-03		
	Total	1.129	23			

Growth response of juvenile *Scylla serrata* fed formulated diets with graded protein levels

		Sum of Squares	df	Mean Square	F	Sig.
SGR	Between Groups	67.628	5	13.526	114.182	.000
	Within Groups	3.554	30	.118		
	Total	71.182	35			
FCR	Between Groups	90.433	5	18.087	301.772	.000
	Within Groups	1.798	30	5.993E-02		
	Total	92.231	35			
PER	Between Groups	1.980	5	.396	83.300	.000
	Within Groups	.143	30	4.755E-03		
	Total	2.123	35			
WETWG	Between Groups	21437877.664	5	4287575.533	363.611	.000
	Within Groups	353749.775	30	11791.659		
	Total	21791627.439	35			
DRYWG	Between Groups	23025271.669	5	4605054.334	446.580	.000
	Within Groups	309354.567	30	10311.819		
	Total	23334626.236	35			
CWG	Between Groups	74175.653	5	14835.131	2419.297	.000
	Within Groups	183.960	30	6.132		
	Total	74359.613	35			
MF	Between Groups	281.988	5	56.398	1443.296	.000
	Within Groups	1.172	30	3.908E-02		
	Total	283.160	35			

Proximate composition of juvenile *Scylla serrata* fed formulated diets with graded protein levels

		Sum of Squares	df	Mean Square	F	Sig.
M	Between Groups	304.598	7	43.514	129.130	.000
	Within Groups	5.392	16	.337		
	Total	309.989	23			
DM	Between Groups	304.598	7	43.514	129.130	.000
	Within Groups	5.392	16	.337		
	Total	309.989	23			
CP	Between Groups	82.154	7	11.736	199.767	.000
	Within Groups	.940	16	5.875E-02		
	Total	83.094	23			
EE	Between Groups	19.650	7	2.807	182.628	.000
	Within Groups	.246	16	1.537E-02		
	Total	19.896	23			
CF	Between Groups	8.663	7	1.238	21.085	.000
	Within Groups	.939	16	5.870E-02		
	Total	9.602	23			
CA	Between Groups	109.152	7	15.593	46.237	.000
	Within Groups	5.396	16	.337		
	Total	114.548	23			
NFE	Between Groups	9.107	7	1.301	84.641	.000
	Within Groups	.246	16	1.537E-02		
	Total	9.353	23			
GE	Between Groups	1.080	7	.154	50.701	.000
	Within Groups	4.868E-02	16	3.043E-03		
	Total	1.129	23			

Growth response of juvenile *Scylla serrata* fed formulated diets with lipid supplements

		Sum of Squares	df	Mean Square	F	Sig.
SGR	Between Groups	16.109	5	3.222	211.244	.000
	Within Groups	.458	30	1.525E-02		
	Total	16.567	35			
FCR	Between Groups	.148	5	2.967E-02	31.565	.000
	Within Groups	2.820E-02	30	9.400E-04		
	Total	.177	35			
PER	Between Groups	.395	5	7.890E-02	30.801	.000
	Within Groups	7.685E-02	30	2.562E-03		
	Total	.471	35			
WETWG	Between Groups	6799579.018	5	1359915.804	54.925	.000
	Within Groups	742790.602	30	24759.687		
	Total	7542369.620	35			
DRYWG	Between Groups	12751942.723	5	2550388.545	537.394	.000
	Within Groups	142375.474	30	4745.849		
	Total	12894318.197	35			
CWG	Between Groups	1531.283	5	306.257	14.146	.000
	Within Groups	649.477	30	21.649		
	Total	2180.759	35			
MF	Between Groups	83.114	5	16.623	321.558	.000
	Within Groups	1.551	30	5.169E-02		
	Total	84.665	35			